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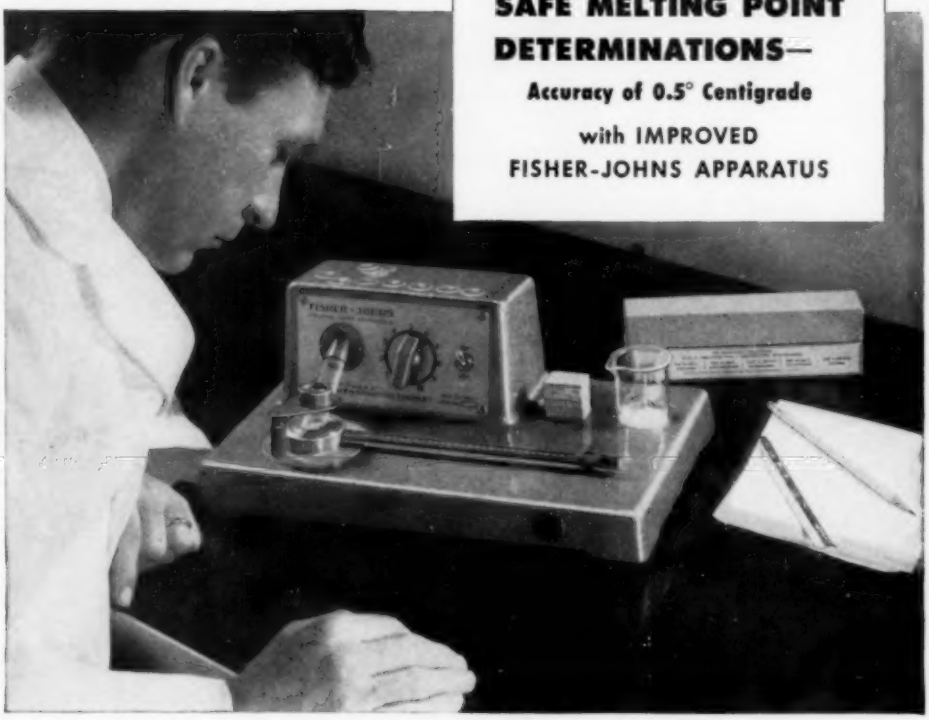

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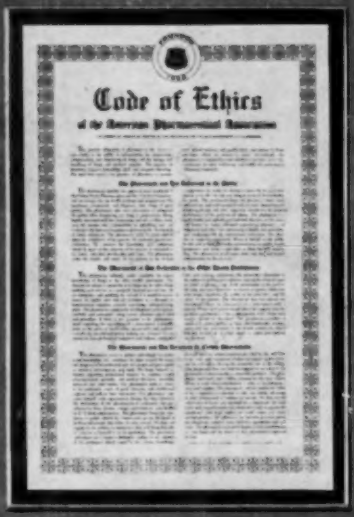

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME XLVI — *FEBRUARY 1957* — NUMBER 2 — CONSECUTIVE NO. 4

Central Stimulating Properties of Some Terpenones*

By DUANE G. WENZEL† and CHARLES R. ROSS

Dicyclic and monocyclic terpenones were studied for their central stimulating effects. Differences in the convulsive actions were noted for the two types of terpenones and a structure-activity relationship is postulated to explain variations in toxicity. Tests for arousal of hexobarbitalized mice, respiratory stimulation, running movement, and total movement did not indicate any consistent differences between the two groups. The possible advantage of some terpenones as convulsants for the screening of petit mal anticonvulsants is studied and discussed.

DESPITE THE FACT that camphor and thujone are among the oldest known stimulants of the central nervous system, they are seldom used for this purpose. As clinical analeptics their action has been generally unreliable (1).

This investigation was initiated for the following reasons: a literature survey revealed no overall study of the central effects of camphor or thujone; preliminary studies in our laboratories indicated that these agents produced an intense and prolonged stimulation; and the related natural terpenones, both monocyclic and dicyclic, have not been examined for their central action.

Although correlation between anti-Metrazol® activity and the clinical value of a drug for petit mal is not always good, chemoshock is still a valuable screening technique. As a corollary of the initial phase of the investigation the most actively convulsant terpenones were tested to determine if they offered any advantages over Metrazol as a means of evaluating anticonvulsants.

* Received June 15, 1956, from the School of Pharmacy, University of Kansas, Lawrence.

Abstracted from a portion of the data contained in a thesis submitted to the Graduate School of the University of Kansas by Charles R. Ross in partial fulfillment of the requirements for the degree of Master of Science.

† Present address: School of Pharmacy, University of Kansas, Lawrence.

EXPERIMENTAL AND RESULTS

Materials.—Menthone, piperitone, pulegone, and carvone were furnished by Givaudan-Delawanna, and ionone, a mixture of the α- and β-isomers, by the Verona Chemical Company. The camphor was a commerical synthetic product. Thujone, fenchone, dihydrocarvone, pinocamphone, and umbellulone were isolated from the appropriate volatile oils according to methods readily available in the literature.

Convulsive and Lethal Doses.—All values were obtained using male albino mice weighing from 18 to 25 Gm. The terpenones were administered to groups of 10 mice per dose with a geometric progression of 1.1. Results were evaluated graphically according to Litchfield and Wilcoxon (2). The terpenones first were injected subcutaneously as propylene glycol solutions and convulsions were delayed from six to eight hours after administration. When sesame oil was used as the solvent convulsions usually occurred within one-half hour. All tests were made with the oil as the vehicle.

The CD_{50} values were determined for all compounds which produced convulsions without killing. As a number of the terpenones were lethal at the convulsive dose level, LD_{50} values were obtained with these compounds.

Examination of Table I and the chemical structures of the terpenones reveals that with the exception of umbellulone all the dicyclic compounds caused convulsions at nonlethal doses. The characteristic effect of the monocyclic terpenones and

TABLE I.—CONVULSIVE, LETHAL, AND AROUSAL VALUES OF TERPENONES FOR MICE

Compound[a]	CD_{50}[b] mg./Kg.	LD_{50}[b]	Sleeping Time[c] Seconds ± S. E.
Control[d]	.....	.....	2443 ± 36
Sesame oil	.....	.....	2559 ± 35
Camphor	600 (506–723)	.....	1894 ± 35
Fenchone	1133 (1030–1246)	.....	1161 ± 55
Pinocamphone	585 (480–693)	.....	1119 ± 77
Thujone	590 (475–705)	.....	967 ± 60
Umbellulone	.....	965 (784–1187)	1209 ± 39
Carvone	.....	2675 (2075–3478)	1564 ± 96
Dihydrocarvone	.....	2900 (2180–3857)	1050 ± 35
Ionone	.....	2605 (2113–3198)	722 ± 91
Menthone	.....	2180 (1929–2463)	1317 ± 42
Piperitone	.....	1420 (1203–1676)	1218 ± 23
Pulegone	.....	1709 (1428–2023)	1918 ± 29

[a] Ten mice per dose.
[b] Figures in parentheses are 95% confidence limits.
[c] Hexobarbital sodium, 100 mg./Kg. i. p., followed in five minutes by 500 mg./Kg. of the terpenone subcutaneously.
[d] Hexobarbital sodium, 100 mg./Kg.

umbellulone was extreme excitement followed in rapid order by convulsions, respiratory depression, and death. The convulsions produced by both mono- and dicyclic terpenones were predominantly clonic in character becoming tonic or with a tonic component in lethal doses.

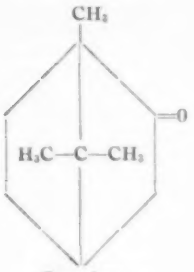
Arousal Action.—Tests were conducted with mice of the same weight range and group size as in the preceding experiment. Hexobarbital sodium was injected i. p. in a dose of 100 mg./Kg. This was followed in five minutes by the subcutaneous administration of 500 mg./Kg. of the terpenone. Sesame oil was used as the vehicle as propylene glycol not only delayed the effect of the terpenone as previously indicated, but in a control group with this vehicle alone actually prolonged the sleeping time. Several trials were also made administering the sesame oil solution i. p., but no significant difference was noted from the sleeping time with subcutaneous administration. Two control groups were used, one with hexobarbital alone and the other with hexobarbital followed by sesame oil in an equivalent volume to that administered with the terpenones. The end point of the sleeping time was taken when the mouse was sufficiently coordinated to climb out of a small metal box into which air was blown as a means of motivating activity. This apparatus has been previously described (3).

The mean sleeping times as listed in Table I were compared by an analysis of variance (4). The following combinations had a P value of less than 0.01: monocyclic compounds to control; dicyclic compounds to control; thujone to control; thujone to the other dicyclic compounds; and thujone to the monocyclic compounds. Results were at least as significant when ionone was substituted for thujone. Ionone, the most active awakening agent, was somewhat more effective than thujone ($P = 0.05$). A comparison of the dicyclic and monocyclic terpenones revealed no significant difference.

Effect on Pentobarbital-Depressed Respiration.—Black-hooded male rats weighing from 200 to 300 Gm. were anesthetized with ether, the trachea cannulated and the cannula attached to sensitive inlet and outlet valves. The inlet valve was connected to a T-tube, one arm of which led to a recording tambour, the other to a bottle with a restricted air intake. The method was essentially that of Gaddum (5). Respiration was depressed by the i. p. administration of 35 mg./Kg. of pentobarbital sodium. Further increments of 5 mg./Kg. were administered as necessary to uniformly decrease respiration. When the desired decrease had been achieved 500 mg./Kg. of the terpenone was injected i. p. An aqueous solution of *d*-amphetamine sulfate, 20 mg./Kg. i. p., was used for the purpose of comparison. Four animals were used to test each compound.

Camphor, ionone, and *d*-amphetamine produced small but consistent increases in the depth of respiration with no significant change in rate. The other terpenones were inactive at this dose level.

Coordinated Movement.—The effect of the terpenones on running or walking activity was determined with a vertically revolving activity drum. Revolutions were tabulated on an automatic counter. Four black-hooded male rats weighing from 200 to 350 Gm. were used for each dose tested. The terpenones in sesame oil, a control of this vehicle and picrotoxin, strychnine, and *d*-amphetamine in aqueous solutions were administered i. p. in the doses indicated in Table II. After administering the compounds the rats were immediately placed in the revolving drums in a quiet, well-lighted room at a temperature of 26–28°. Total cage revolutions per rat were recorded for a four-hour period. The mean



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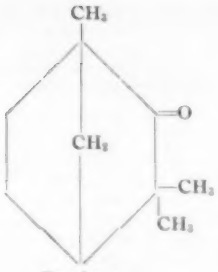

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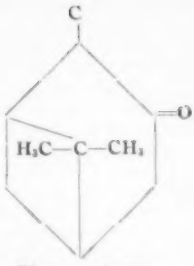
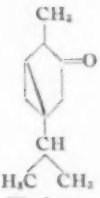


Pinocamphone



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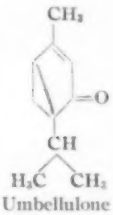

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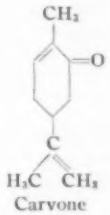

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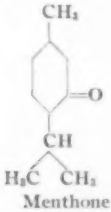
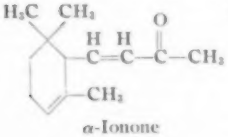
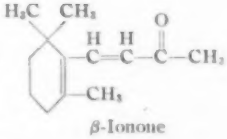
Dihydrocarvone



Menthone



α-Ionone



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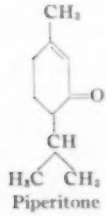

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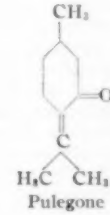

Pulegone

number of revolutions for each dose at which the compounds were tested is recorded in the upper level of figures of Table II.

All of the terpenones caused an increase in running activity over that of the control group. Several induced peak activity at 400 mg./Kg., the highest dose tested. It is likely that a further increase in the size of the dose of these compounds would produce still more activity. There were no obvious differences in the effects of the monocyclic and dicyclic terpenones as groups. It is noteworthy, however, that camphor, as in the arousal test, was a surprisingly poor stimulant up to the maximal dose of 400 mg./Kg. while the monocyclic menthone induced marked activity at 100 mg./Kg.

Total Movement.—The spring-suspended cage of Schulte (6) was used to measure total movement.[1] This apparatus records all motion including tremor and convulsions as well as voluntary activity. A simplification of the usual recording system was accomplished by attaching a light aluminum arm to the wheel of the work adder. Each revolution of the wheel caused the arm to close a microswitch attached to an electric counter. Details of this modification are shown in Fig. 1.

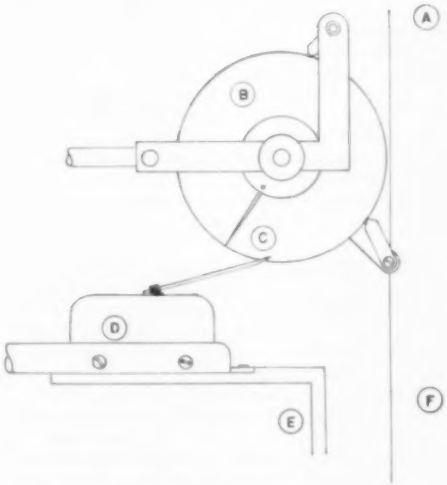

Fig. 1.—Recording mechanism for spring-suspended cage. A - spring connection, B - work adder, C - actuating arm, D - microswitch, E - leads to electric counter, F - cage connection.

The experimental procedure was essentially the same as in the preceding study.

Thujone caused the rats to convulse at doses as low as 100 mg./Kg. and it may be concluded that the CD_{50} of thujone for rats is lower than that for mice (Table I). Because the cages record convulsions as

[1] Thanks are extended to Drs. W. Bass and B. Graham of Upjohn and Company for the springs used in the cages.

TABLE II.—EFFECT OF TERPENONES ON ACTIVITY OF RATS IN REVOLVING AND SPRING-SUSPENDED CAGES

Compound, Dose mg./Kg.	Four Hour Mean per Rat[a] Revolving Cage Revolutions ± S. E. / Spring-Suspended Cage Revolutions ± S. E.			
	50	100	200	400
Camphor	31 ± 9.6	30 ± 6.8	46 ± 2.1	30 ± 7.1
	1.0	1.3	1.8	3.8
Fenchone	24 ± 4.2	37 ± 4.6	121 ± 23.0	148 ± 22.8
	0	0.25	1.0	0.5
Pinocamphone	32 ± 6.1	17 ± 3.7	38 ± 1.8	72 ± 18.4
	2.5	0.75	0	0
Thujone	25 ± 2.1	221 ± 40.3	140 ± 27.2[c]	.....[b]
	0.25	1.0	.....[b]	.....[b]
Umbellulone	37 ± 3.7	65 ± 4.0	63 ± 15.5	98 ± 7.4
	0.5	0.75	0.25	0.25
Carvone	22 ± 7.0	63 ± 4.3	185 ± 10.3	44 ± 15.1
	0	0.5	0.5	1.0
Dihydrocarvone	12 ± 3.4	27 ± 6.0	59 ± 8.3	69 ± 10.1
	0.25	0.75	0	0.5
Ionone	9 ± 3.6	32 ± 6.7	83 ± 9.4	110 ± 9.2
	0	0.75	0.5	0.5
Menthone	19 ± 4.4	277 ± 35.6	200 ± 14.7	166 ± 72.0
	0	0	1.0	1.25
Piperitone	21 ± 4.1	27 ± 8.2	48 ± 9.6	93 ± 6.0
	0.5	0	0	0.25
Pulegone	14 ± 4.8	64 ± 5.2	90 ± 7.0	192 ± 29.5
	0	0.25	0	0
d-Amphetamine sulfate	2	3	4	5
	23 ± 3.5	62 ± 13.7	152 ± 26.0	139 ± 11.2
	31 ± 9.8	38 ± 10.2	44 ± 7.7	51 ± 5.0
Strychnine sulfate	0.1	0.2	0.4	0.8
	14 ± 3.7	19 ± 4.2	23 ± 3.9	34 ± 9.3
	0.25	0.75	0.8	0.5
Picrotoxin	0.2	0.4	0.8	1.6
	61 ± 27.3	51 ± 10.0	114 ± 8.9	17 ± 4.1
	0.25	0	0.5	0.5

[a] Four rats per dose.
[b] Lethal.
[c] Short period of hyperactivity followed by lethal action.

well as other movement, the rats given thujone at 100 mg./Kg. were not placed in the cages until the convulsions had stopped. Examination of the lower level of figures in Table II reveals that of all the compounds tested only *d*-amphetamine induced a marked increase in activity.

Evaluation as Chemoshock Agents.—Trimethadione, phenobarbital sodium, and diphenylhydantoin sodium were evaluated for their ability to modify the convulsions produced by camphor, thujone, pinocamphone, and Metrazol. None of the monocyclic terpenones were tested as their convulsant doses were rapidly lethal.

Each anticonvulsant PD_{50} for mice was determined against the CD_{97} of the convulsants indicated. The CD_{97} of the terpenones as shown in Table III was obtained by a graphic method using the data from the CD_{50} determinations (2). The CD_{97} of Metrazol, 85 mg./Kg., was the value obtained by Swinyard (7). Anticonvulsants were administered

TABLE III.—TERPENONES AS CHEMOSHOCK AGENTS FOR TESTING ANTICONVULSANTS WITH MICE[a]

Convulsant	CD_{97}[b] mg./Kg.	PD_{50} mg./Kg.[b] Phenobarbital Sodium	PD_{50} mg./Kg.[b] Trimethadione
Metrazol[a]	85[c]	40.6 (31.6–76.2)	131.2 (73.0–263.6)
Camphor	815 (648–993)	31.0 (22.4–42.8)	343.5 (270.5–293.4)
Pinocamphone	770 (621–875)	40.8 (27.0–38.4)	286.3 (224.7–363.6)
Thujone	775 (604–887)	30.5 (28.3–48.3)	357.8 (168.9–259.5)

[a] Eight mice per dose.
[b] Figures in parentheses are 95% confidence limits.
[c] Reported by Swinyard, *et al.* (7).

orally to male albino mice weighing from 18 to 25 Gm. Animals were fasted for eight hours prior to the administration. Eight mice were used for each dose tested. Convulsants were administered subcutaneously ten minutes before the anticipated peak anticonvulsant action. Metrazol was given as the aqueous solution, the triterpenes in sesame oil. Times of peak action of the anticonvulsants were those previously reported: trimethadione, one and one-half hours; phenobarbital, two hours; and diphenylhydantoin, three hours (7). The criterion of the test was the ability of the anticonvulsant to prevent both tonic hindleg extension and clonic spasm lasting for more than five seconds over an observation period of two hours. As a rough test a chemoshock agent for the testing of petit mal anticonvulsants should compare favorably to Metrazol in the following respects: clonic convulsions effectively prevented by trimethadione; phenobarbital somewhat less active; and diphenylhydantoin ineffective (8).

The results in Table III include values only for trimethadione and phenobarbital sodium. Diphenylhydantoin sodium did not exert a protective effect at nontoxic doses which is in accordance with its lack of activity for petit mal.

DISCUSSION

Except for differences observed in the CD_{50} and LD_{50} determinations, there was little to distinguish between the monocyclic or dicyclic terpenones as separate groups. The monocyclic compounds, without exception, were lethal at convulsive dose levels. This was also true of umbellulone, a dicyclic terpenone. A structural feature common to umbellulone and the more toxic of the monocyclic terpenones is adjacent carbonyl and isopropyl groups. In the process of biotransformation a terpenone is first reduced to the corresponding alcohol, conjugated with glycuronic acid and then excreted as the conjugate (9). The terpinols are generally less toxic than the terpenones and as observed with the isomers of menthol, toxicity is indirectly related to the ease of glucuronide formation (9). On these bases it is postulated that the toxicity of the terpenones is increased by steric hindrance of the carbonyl group which prevents biotransformation to less toxic compounds. The predominantly clonic nature of the convulsions indicates stimulation of the higher centers.

There was no apparent correlation between the convulsant or lethal doses and the arousal activity of the terpenones. While thujone was very active in both of these tests, camphor which was as effective a convulsant as thujone gave poor results as an arousal agent. The arousal action of camphor is frequently attributed to the pain stimuli produced by the injection; however, it would be difficult to relate the marked activity of thujone and ionone to this effect.

The terpenones were almost uniformly ineffective as respiratory stimulants. Only camphor, ionone, and the *d*-amphetamine control produced a consistent stimulation. In view of the fact that with the exception of ionone these results are not in accord with the arousal test, it would appear that the respiratory center does not necessarily share the terpenones' stimulation of the higher centers which cause awakening. As only one dose of the terpenones, 500 mg./Kg., was tested in this experiment it is possible that higher doses of the inactive compounds may be effective.

In the dose range tested for coordinated activity, 50 to 400 mg./Kg., several of the compounds reached peak activity at an intermediate dose and running movement was decreased at higher levels. This decrease in activity may be the result of the stimulation spreading to the cord as it had been observed in the first experiment that the convulsions were clonic, but at higher doses or before death became tonic or had a tonic component.

The terpenones had little activity in the spring-suspended cage test resembling strychnine and picrotoxin. As this test is believed to measure cortical stimulation (6) and as most of the terpenones were active arousal agents it may be concluded that the arousal action was the result of subcortical stimulation. Camphor which fared poorly in the awakening test was the most active of the terpenones in the spring-suspended cage test. It appears that the site of camphor's central stimulation must differ in certain respects from the terpenones as a whole.

Trimethadione controlled Metrazol convulsions better than those induced by the terpenones tested. On this basis Metrazol apparently offers a more sensitive means of screening potential anticonvulsants than do the terpenones. It should be pointed out, however, that both the speed of onset and duration of convulsions differed markedly for Metrazol and the terpenones. The convulsive action of the former was observed within five to fifteen minutes of its administration and lasted for only ten to fifteen minutes. The terpenones required from ten to ninety minutes to produce convulsions which then lasted for at least twenty to sixty minutes. It is probable that they were often producing maximal convulsive activity when the level of trimethadione was declining. This prolonged action of the terpenones may be a distinct advantage over the shorter Metrazol seizures. Effective protection for Metrazol convulsions requires a relatively short duration of action while the terpenones provide a more rigorous test.

SUMMARY

1. Eleven terpenones were investigated for their activity on the central nervous system.

2. The CD_{50} values are given for the dicyclic compounds except umbellulone and with this exception the dicyclic terpenones induced clonic convulsions at nonlethal doses. The monocyclic terpenones and umbellulone were lethal at convulsive dose levels. LD_{50} values are presented for these compounds. A postulated structure to activity relationship is advanced to explain certain observed differences in toxicity.

3. Both a dicyclic (thujone) and a monocyclic (ionone) terpenone produced highly significant reductions in the sleeping times of hexobarbitalized mice. While most of the terpenones were

effective arousal agents, camphor demonstrated little, if any activity.

4. The respiratory depth of pentobarbital-depressed rats was slightly increased by camphor, ionone, and the *d*-amphetamine control.

5. Most of the terpenones caused a marked increase in the running activity of rats yet total activity as measured by the spring-suspended cage was negligible, camphor causing the greatest increase of the test compounds.

6. Camphor, pinocamphone, and thujone were compared to Metrazol as convulsants for screening petit mal anticonvulsants. While trimethadione provided better Metrazol protection than against the terpenones, the latter were active over a longer period. This greater duration of action may be advantageous.

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The Effect of *l*-Menthol on the Systemic Blood Pressure*

By NATHAN RAKIETEN and MORRIS L. RAKIETEN

_l_-Menthol in Propylene glycol produces a fall in blood pressure of cats and rabbits when administered i.v. in dosages of 0.25 mg./Kg. and above. When mixtures of _l_-menthol in propylene glycol and nicotine are administered i. v. to cats and rabbits the vasoconstriction produced by nicotine is blocked completely when the ratio of menthol to nicotine is 40 to 80. The degree of vasoconstriction is decreased 10 to 60 per cent when the ratio of menthol to nicotine is 6 to 20.

PRELIMINARY STUDIES made by us (1) on humans suggested that the inhalation of menthol vapor produced a transient vasodilatation of the nasal mucosa in some subjects; in others equivocal results were obtained. Macht (2) previously reported that the intravenous administration of *l*-menthol in alcohol produced a fall in the systemic blood pressure of cats. This is the only reference concerning such an action of menthol that was found in the literature.

The vasodilatation observed in our subjects followed the inhalation of menthol vapor; hence, we believed that it would be of interest to reinvestigate the alteration in systemic blood pressure following its intravenous administration to cats and rabbits. When, in the course of these experiments, it was noted that menthol produced a systemic vasodilatation, as indicated by a fall in blood pressure, its effect on the vasoconstrictor action of nicotine was also investigated.

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This study was supported by a grant from the Brown and Williamson Tobacco Corporation, Louisville, Ky.

EXPERIMENTAL

Thirteen rabbits and eight cats were used in this study. Sodium phenobarbital, 140 mg./Kg. i. p., was used to anesthetize the cats and pentobarbital sodium, 35 mg./Kg. i. v., the rabbits. Blood pressure was recorded using an ink writing system by the traditional arterial cannulation procedure. Five per cent sodium citrate was used as the anticoagulant.

l-Menthol, U. S. P., in propylene glycol was administered intravenously via the femoral vein (in a few instances via the marginal ear vein of the rabbit) in concentrations of 0.4 through 10%. Dosages of 0.1 through 4.0 mg./Kg. of menthol were given to the cats and 0.25 through 18.5 mg./Kg. to the rabbits, with the vast majority falling within the range of 0.25 through 2.0 mg./Kg. Nicotine in aqueous solution varying from 0.025 through 0.4% was administered i. v. The dosages of nicotine used to produce vasoconstriction in cats varied from 0.006 through 0.1 mg./Kg. (0.5 mg./Kg. in one experiment) and in rabbits 0.25 through 2 mg./Kg.

The antagonistic effect of menthol on nicotine was studied by administering the drugs at separate times and in most of the cat experiments simultaneously by premixing the dosages of the drugs in the same syringe. Usually the drugs were given at five- to ten-minute intervals and occasionally at three-

minute intervals, but in no instance was another dosage given before the blood pressure had returned to its original level or reached a new steady state.

RESULTS

l-Menthol, U. S. P., in propylene glycol when given in dosages of 0.25 mg./Kg. and above, produced a fall in blood pressure in both cats and rabbits. The fall in blood pressure produced by *l*-menthol is most likely due to a vasodilatation. Typical responses in the cat and rabbit are shown in Figs. 1 and 2 and Table I. As indicated in Fig. 2

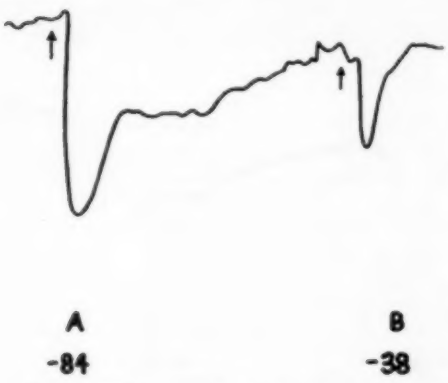

Fig. 1.—Fall in systemic blood pressure of the cat following i. v. administration of 2.0 mg./Kg. of *l*-menthol (A) and following an equivolume of propylene glycol, 0.3 cc. (B). Time between A and B was 3 minutes.

the depressor action of *l*-menthol in propylene glycol may be followed by a pressor response when dosages up to 0.5 mg./Kg. are administered to rabbits. Occasionally such a response was also seen in cats. However, when the dose of *l*-menthol was above 0.5 mg./Kg. only a fall in blood pressure occurred.

Propylene glycol also produces a fall in blood pressure; however, the effects ascribed to menthol are real, since *l*-menthol in propylene glycol produces

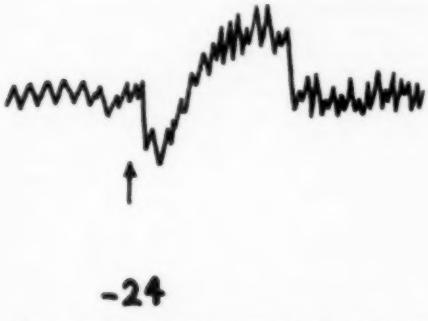

Fig. 2—Fall in blood pressure of rabbit following i. v. dose of 0.5 mg./Kg. of *l*-menthol in propylene glycol. Depressor action lasted 30 seconds.

at least a 40 through 60% greater fall in blood pressure than equal volumes of propylene glycol and its duration of action is longer.

Further, menthol in 50% alcohol, 0.4 mg./Kg., produces a fall in blood pressure which is equal to the same dose administered in propylene glycol, whereas equal volumes of 50% alcohol have no effect on the blood pressure (Fig. 3). These findings are in accordance with those described by Macht (2).

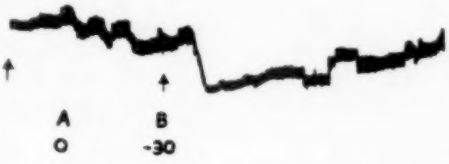

Fig. 3.—Fall in blood pressure of cat following i. v. administration of 0.4 mg./Kg. of *l*-menthol in 50 per cent ethyl alcohol (B) and no change following an equivolume of 50 per cent ethyl alcohol, 0.2 cc. (A). Time between A and B was 5 minutes.

Antagonistic Action of *l*-Menthol on Nicotine.—*l*-Menthol in propylene glycol completely blocks the vasoconstrictor action of nicotine when mixtures of menthol and nicotine containing 40 to 80 times more menthol than nicotine are administered intravenously in cats and rabbits (Fig. 4). When the relationship of menthol to nicotine is 8 through 20 times, a 10 to 60% decrease in vasoconstrictor action of nicotine was noted. Usually this was directly proportional to the amount of menthol given in excess of nicotine. Propylene glycol in comparable volumes as those given with menthol when given with nicotine does not alter the vasoconstrictor action of nicotine (Figs. 4 and 5). In a few instances in rabbits the vasoconstrictor action of 0.5 and 1.0 mg./Kg. dosages of nicotine have been completely negated by the previous administration of 1.5 and 3.0 mg./Kg. dosages of *l*-menthol in propylene glycol.

DISCUSSION

The i. v. administration of *l*-menthol in propylene glycol in dosages of 0.25 mg./Kg. and above produces a fall in the systemic blood pressure of rabbits

TABLE I.—TYPICAL FALLS IN SYSTEMIC BLOOD PRESSURE PRODUCED BY THE I. V. ADMINISTRATION OF MENTHOL IN PROPYLENE GLYCOL IN CATS AND RABBITS

Cats		Rabbits	
Dose Mg./Kg.	Fall in Blood Pressure	Dose Mg./Kg.	Fall in Blood Pressure
0.2	−6	0.25	−14
0.3	−8	...	..
0.4	−25	...	..
0.5	−32	0.5	−16
0.6	−42	...	..
0.8	−38	...	..
1.0	−36	...	..
2.0	−49	2.0	−49
4.0	−69	...	..
..	..	7.4	−32
..	..	18.6	−68

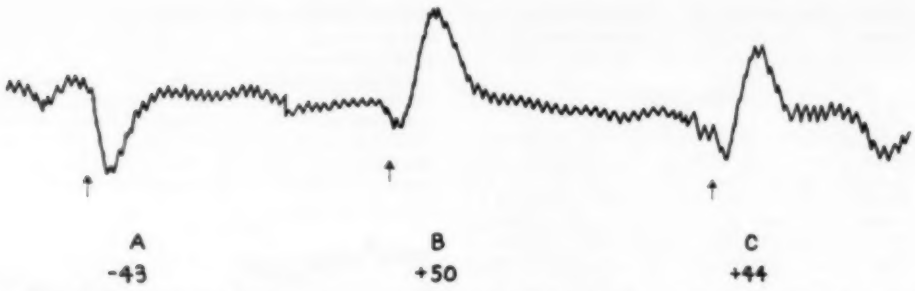

Fig. 4.—Complete block of vasoconstrictor action of 0.016 mg./Kg. of nicotine by a 0.75 mg./Kg. dose of *l*-menthol in propylene glycol (A), contrasted with action of nicotine (B) and nicotine plus propylene glycol (C) in a cat. All doses given i. v. Time between A and B was 6 minutes, between B and C—5 minutes.

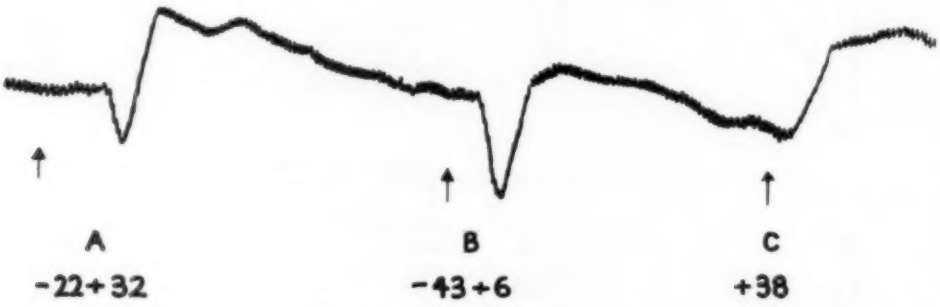

Fig. 5.—Antagonistic action of *l*-menthol in propylene glycol, 0.4 mg./Kg. on a 0.048 mg./Kg. dose of nicotine in a cat (B) contrasted with the action of 0.048 mg./Kg. of nicotine (C) and 0.048 mg./Kg. of nicotine plus propylene glycol (A) in a cat. All doses given i. v. Time between A and B was 5 minutes, between B and C—5 minutes.

and cats. These findings confirm the observations previously reported by Macht (2) following the i. v. administration of alcoholic solutions of menthol to cats. Large dosages of menthol are necessary to produce a fall in systemic blood pressure and such dosages cannot be obtained by using saturated aqueous solutions or from the inhalation of menthol vapor for periods of up to one hour.

When nicotine and menthol are administered simultaneously in ratios of 1:40 and 1:80, the vasoconstriction ordinarily produced by nicotine is not seen and the vasodilating action of menthol predominates. The mechanism of this action of menthol has not been investigated.

SUMMARY AND CONCLUSIONS

1. *l*-Menthol in propylene glycol produces a fall in the systemic blood pressure of cats and rabbits when given intravenously in dosages of 0.25 mg./Kg. and above.

2. *l*-Menthol in propylene glycol when given intravenously, simultaneously with nicotine, to both cats and rabbits either decreases the vasoconstrictor action of nicotine or blocks it completely depending upon the amount of menthol given. When the relationship of menthol to nicotine is 40 to 80 times, a complete block is obtained; when the relationship is 8 to 20 times, the decrease varies from 10 to 60 per cent.

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Determination of Sympathomimetic Amines by Ion Exchange*

By MURIEL C. VINCENT,† EDWARD KRUPSKI, and LOUIS FISCHER

A convenient ion exchange method has been developed for the quantitative determination of sympathomimetic amines and some of their pharmaceutical preparations. It was found that the strongly basic anion exchange resin, Amberlite IRA-400, gave satisfactory results except when used with the amines containing phenolic groups. The carboxylic cation exchange resin, Amberlite IRC-50, was used successfully in the quantitative determination of the sympathomimetic amines, including the latter group, and their pharmaceutical preparations providing no sodium ions or other basic interfering substances were present. Moreover, the weakly basic anion exchange resin, Amberlite IR-45, was found suitable for the quantitative separation of all the amines used in this work including those containing phenolic groups and in in the presence of sodium ions or other interfering substances.

THE SYMPATHOMIMETIC AMINES have been determined quantitatively by a number of different assay procedures; however, none of these methods have been found applicable to all sympathomimetic amines and their pharmaceutical preparations. Since the chemical nature of these compounds is such that they lend themselves to ion exchange, experiments were undertaken to develop a method which could be applied to the various sympathomimetic amines and their pharmaceutical products.

EXPERIMENTAL

The experimental work of this investigation involved three studies: (*a*) the quantitative recovery of the sympathomimetic amines from solutions of their salts by means of the strongly basic anion exchange resin Amberlite® IRA-400,[1] (*b*) their recovery from solutions and pharmaceutical preparations by means of carboxylic acid type cation exchange resin Amberlite IRC-50, and (*c*) the recoveries obtained employing the weakly basic anion exchange resin Amberlite IR-45 with solutions of the amines and their medicinal preparations.

The experiments with Amberlite IRA-400 indicated that this resin was not suitable for the sympathomimetic amines possessing phenolic groups. Amberlite IRC-50 was satisfactorily utilized with solutions of sympathomimetic amines but not with their pharmaceutical preparations containing sodium ions and other interfering substances. The following results are those obtained with the use of Amberlite IR-45.

Amine Salts.—The procedure as outlined previously by the authors (1) was followed. Exceptions to the procedure were observed in several instances. Due to the volatility of methylhexaneamine this substance was weighed into a small covered beaker containing 2 ml. of 0.1 *N* hydrochloric acid, and this solution was poured into the column of resin. The filtrate was collected in a flask containing 5 ml. of 0.1 *N* hydrochloric acid, and the excess acid was determined by back titration with 0.1 *N* sodium hydroxide. The quantity of amine was then calculated from the number of ml. of HCl consumed by the reaction.

Epinephrine was dissolved in water with the aid of 3 ml. of 0.1 *N* hydrochloric acid and then treated as the other solutions of amines. In the case of the easily oxidized amines, isopropylarterenol and epinephrine, it was necessary to buffer the regenerated resin before the amine solution was passed through the columns. The buffering was accomplished by passage of 30 ml. of Mac Ilvaine's buffer mixture *p*H 5 (10.3 ml. 0.2 *M* Na_2HPO_4 and 9.7 ml. of 0.1 *M* citric acid) followed by about 100 ml. of boiled and cooled distilled water to remove excess buffer. It was also necessary to cover the column and receiving flasks with aluminum foil to minimize the action of light upon the compounds. The filtrate was collected in a flask containing 5 ml. of 0.1 *N* hydrochloric acid, and the excess acid was determined by back titration with 0.1 *N* sodium hydroxide. Blanks were run with all determinations.

The quantitative recoveries of the sympathomimetic amines through the use of Amberlite IR-45 are given in Table I.

Liquids.—A sample containing about 25 mg. of each sympathomimetic amine was passed through a column of regenerated Amberlite IR-45. If the liquid was a viscous elixir or syrup, it was found best to dilute the sample with distilled water to a volume of 100 ml. to facilitate the exchange procedure. The resin was rinsed with 100 ml. of distilled water to remove sodium and other interfering constituents and the washings were rejected. Then the resin was washed with 200 ml. of 75% ethyl alcohol to remove the amine, and the filtrate was collected and titrated with 0.1 *N* hydrochloric acid using methyl red T. S. as indicator.

Capsules and Tablets.—The contents of a suitable number of tablets or capsules was dissolved in distilled water. The solution was filtered, the filter rinsed with distilled water, and the filtrate made up to volume. The procedure as outlined above for liquids was used on an aliquot of the combined solution equivalent to about 25 mg. of the amine.

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† Present address: North Dakota Agricultural College, School of Pharmacy, Fargo.
[1] Rhom and Haas Company, Resinous Products Division, Philadelphia, Pa.

TABLE I.—RECOVERY OF AMINES WITH AMBERLITE IR-45

Amine	Recovery, %
Dextroamphetamine sulfate	99.8
Amphetamine sulfate	99.8
Aramine hydrogen tartrate	98.7
Cyclopentamine hydrochloride	98.2
Ephedrine sulfate	99.4
Epinephrine	101.1
Hydroxyamphetamine hydrobromide	99.4
Isopropylarterenol hydrochloride	100.2
Methamphetamine hydrochloride	99.9
dl-Methamphetamine hydrochloride	99.5
Methoxyphenamine hydrochloride	99.6
Methylhexaneamine	100.6
Naphazoline hydrochloride	99.7
Phenylephrine hydrochloride	99.7
Phenylpropanolamine hydrochloride	99.6
Phenylpropylmethylamine hydrochloride	99.2
Racephedrine sulfate	99.8
Tuaminoheptane sulfate	97.9

Jellies.—The contents of one or more tubes of jelly as was necessary to obtain a sufficient amount of amine was weighed. The jelly was diluted to 100 ml. with distilled water, the solution was filtered, the filter washed with distilled water, and the filtrate made up to volume. The procedure as outlined above for liquids was used on an aliquot of the combined solution equivalent to about 25 mg. of the amine.

Suspensions.—The preparation was filtered, the filter washed with distilled water, and the filtrate made up to volume. The method as given above for liquids was used on an aliquot of the combined solution equivalent to about 25 mg. of the amine.

Inhalers.—The cotton inhaler plug was removed from the plastic container and placed in a volumetric flask. Twenty-five milliliters of ethyl alcohol and 25 ml. of 0.1 *N* hydrochloric acid were added, the mixture was shaken and allowed to stand for several hours. The cotton plug was then removed and washed with a mixture of ethyl alcohol and 0.1 *N* hydrochloric acid and the washings transferred to the flask. The liquid was made up to volume with alcohol, and an aliquot of the solution equivalent to about 25 mg. of the amine was then passed through the column of ion exchange resin. The resin was rinsed with 200 ml. of 75% ethyl alcohol, and eluate was collected in a flask containing 5 ml. of 0.1 *N* hydrochloric acid. The excess acid was titrated with 0.1 *N* sodium hydroxide using methyl red T. S. as indicator.

Emulsions.—An accurately measured portion of the emulsion was transferred to a 100-ml. volumetric flask, 50 ml. of ethyl alcohol was added, the mixture was filtered, washed, and sufficient alcohol added to bring the volume to 100 ml. An aliquot representing about 25 mg. of the drug was evaporated and the residue was taken up with 25 ml. of distilled water. The method as given above for liquids was then employed.

Oily Solutions.—An accurately measured or weighed portion of the preparation (ephedrine spray) was placed in a 100-ml. volumetric flask, 50 ml. of distilled water was added, and the mixture was shaken and allowed to stand for several hours. The oily portion was separated, and the aqueous portion was made up to a volume of 100 ml. by the addition of distilled water. An aliquot representing about 25 mg. of the amine was passed through the column of resin according to the procedure above for liquids.

Epinephrine and Isopropylarterenol Preparations.—The procedure for these preparations was the same as for general liquids except that the columns were buffered before passage of the amine solution through the resin, aluminum foil was placed around the apparatus to exclude light, and 5 ml. of 0.1 *N* hydrochloric acid was added to the receiving flask.

In Table II are listed the results obtained in the recovery of the amines from their various dosage forms. The dosage forms were prepared to resemble commercially prepared products of these amines.

DISCUSSION

The strongly basic anion exchanger Amberlite IRA-400 gives satisfactory results except with amines containing phenolic groups (Aramine® hydrogen tartrate, *l*-epinephrine, hydroxyamphetamine hydrobromide, isopropylarterenol hydrochloride, phenylephrine hydrochloride). The resin binds the phenolic compounds so strongly that they cannot be removed from the resin column under the conditions of the proposed assay. The strong basicity of the resin results in decomposition of the easily oxidized amines, isopropylarterenol and epinephrine.

Amberlite IRC-50 can be used to a good advantage in assaying solutions of pure amine salts. It is not effective with preparations containing sodium ions or other interfering substances as they are removed from the column simultaneously with the amines upon addition of hydrochloric acid.

The use of Amberlite IR-45, a weakly basic anion exchange resin, is recommended for an assay procedure of the sympathomimetic amines and their preparations which do not contain local anesthetics and antihistamines. The resin can be used successfully with both phenolic and nonphenolic compounds, as both types of compounds are held weakly to the resin structure. With proper buffering of the resin columns and wrapping with aluminum foil, the method can be applied to those amines which are easily decomposed in the presence of alkali and light. Separation of water-soluble interfering basic constituents in pharmaceutical preparations is readily achieved by rinsing the resin column with water. It is assumed that this is due to a greater attraction of sodium ions for the more polar solvent water than for the resin, thus leaving the amines to be removed from the resin with a less polar solvent ethyl alcohol.

The results obtained by this method indicate that a simplified method yielding quantitative results can be applied to all the amines employed in this study. Normally several different assay procedures are required depending upon the nature of the amine and dosage form being determined.

The proposed method is also applicable to preparations containing other medicinal agents in conjunction with sympathomimetic amines and offers an effective and complete separation of the various medicinal agents.

The ion exchange method is no more time con-

TABLE II.—RECOVERY OF SYMPATHOMIMETIC AMINES FROM DOSAGE FORMS

Type of Preparation:	Recovery by Amberlite IR-45, %
Elixir[a]	
Amphetamine sulfate 0.05%	100.3
Dextro amphetamine sulfate 0.1% with vitamin B_1, sodium and potassium glycerophosphates	102.0
Methamphetamine hydrochloride 0.07%	98.3
Phenylephrine hydrochloride 0.1%	98.8
Phenylpropanolamine hydrochloride 0.4%	100.5
Solution	
Amphetamine 0.1% in liquid petrolatum	99.6
Cyclopentamine hydrochloride 0.5% in normal saline	100.5
Ephedrine 1% in liquid petrolatum	97.4
Ephedrine sulfate 1% in isotonic dextrose solution	100.4
Epinephrine hydrochloride 0.1% in normal saline	99.0
Methamphetamine hydrochloride 0.25% with sodium sulfacetamide 2.5% in normal saline	97.9
dl-Methamphetamine hydrochloride 0.1% with sodium sulfathiazole 1% in normal saline	98.2
dl-Methamphetamine hydrochloride 0.25% with bacitracin in normal saline	99.3
Phenylephrine hydrochloride 1% in normal saline	98.8
Phenylpropanolamine hydrochloride 1% in normal saline	100.3
Tuaminoheptane sulfate 1% in normal saline	98.9
Syrup[b]	
Ephedrine hydrochloride 0.22%	99.6
Ephedrine sulfate 0.08% with calcium iodide and pentobarbital sodium	101.2
Methoxyphenamine hydrochloride 1%	99.4
Methoxyphenamine hydrochloride 0.34% with dihydrocodeinone bitartrate, sodium citrate, hyoscyamine hydrobromide	99.6
Phenylephrine hydrochloride 0.13% with codeine phosphate, potassium guaiacolsulfonate, ammonium chloride	99.5
Tablet	
Dextro amphetamine sulfate 5 mg.	101.2
Dextro amphetamine sulfate 5 mg., and caffeine 30 mg.	100.3
Dextro amphetamine sulfate 5 mg., with amobarbital 32 mg., aspirin 0.166 Gm., phenacetin 0.166 Gm.	99.7
Dextro amphetamine sulfate 5 mg., and amobarbital 32 mg.	100.7
Amphetamine sulfate 2.5 mg., with aspirin 0.166 Gm., phenacetin 0.166 Gm.	100.3
Isopropylarterenol hydrochloride 15 mg., and sodium bisulfite 2 mg.	99.2
Hydroxyamphetamine hydrobromide 20 mg.	99.4
dl-Methamphetamine hydrochloride 2.5 mg., with aspirin, 0.227 Gm., acetophenetidin 0.162 Gm.	97.7
Methoxyphenamine hydrochloride 100 mg.	99.5
Capsule	
Ephedrine hydrochloride 25 mg.	100.0
Ephedrine hydrochloride 25 mg., with aminophylline 0.136 Gm., amobarbital 25 mg.	99.7
Ephedrine sulfate 25 mg., with phenobarbital 30 mg.	100.7
Phenylephrine hydrochloride 10 mg.	99.6
Phenylpropanolamine hydrochloride 50 mg.	99.8
Racephedrine hydrochloride 25 mg.	100.4
Jelly[c]	
Ephedrine 1% with butyl amino benzoate 0.66%	99.0
Naphazoline hydrochloride 0.05%	99.5
Phenylephrine hydrochloride 0.5%	99.9
Inhaler	
Methylhexamine 250 mg., with camphor, menthol	99.6
Tuaminoheptane 325 mg., with camphor, menthol	98.7
Emulsion	
Phenylephrine hydrochloride 0.25% with camphor, menthol, mineral oil, acacia	99.8
Suspension	
Tuaminoheptane sulfate 1% with sulfathiazole 5%	100.0
Troche	
Dextro amphetamine sulfate 5 mg., with vitamins A, B_1, B_2, B_6, C, D, nicotinamide	98.0

[a] Aromatic elixir base.
[b] Simple syrup base.
[c] Tragacanth base.

suming than official procedures. When a series of assays is undertaken, a shorter period of time is spent with the ion exchange procedure than with the currently used procedures. As many as 24 determinations have been made in one day. Approximately one hour is required to regenerate the columns prior to use. Approximately two to four hours are needed to remove the amine from the columns depending upon the buffering and washing required before elution of the amine with alcohol.

SUMMARY

1. Three ion exchange resins, Amberlite IRA-400, Amberlite IRC-50, and Amberlite IR-45, were used in the determination of various sympathomimetic amines.

2. Amberlite IR-45 was found to be the most satisfactory resin of the three employed in the recovery studies.

3. The proposed method makes it possible to quantitatively separate various sympathomimetic amines from other medicinal agents when the method is applied to dosage forms.

REFERENCES

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Insoluble Solids as Emulsifiers*

By A. W. JOWDY† and E. A. BRECHT

Twenty-one insoluble solids were screened for suitability in preparing 40 per cent emulsions of liquid petrolatum in water. Seven of these, magnesium hydroxide, magnesium oxide, kaolin, tribasic calcium phosphate, ferric oxide, zinc oxide, and magnesium carbonate, were subjected to comprehensive investigation. The qualities of fresh and aged emulsions were evaluated on the basis of mean globule diameter and interfacial area, centrifugation, oil separation, and viscosity.

THE ABILITY to promote emulsification is possessed by varied classes of compounds. This study was concerned with insoluble solids.

The literature reveals that as early as 1898 an insoluble solid was used as an emulsifying agent. The substance reported was *Tfol*, which contained free gelatinous silica and was used by the Arabs of North Africa as a substitute for soap (1).

Other solids have been investigated as emulsifiers (2–30) and an antagonistic effect between insoluble compounds, similar to that observed among soluble emulsifying agents, has been reported (10).

A measure of the commercial significance of such emulsifiers is reflected by the patents issued to protect specific solids when so employed (31–36).

Mechanism of Emulsification.—Various theories have been advanced principally for the mechanism of emulsification with soluble emulsifiers. These may be listed as the Phase-Volume Theory, The Interfacial Tension Theory, The Adsorbed Film Theory, and The Oriented Wedge Theory.

For insoluble emulsifiers, Finkle, Draper, and Hildebrand (37) believed that both liquids wetted the agent with a finite angle of contact of the interface with the solid. In general one liquid wetted the solid to a greater degree than the other so that the particles were drawn largely into the former. If there were sufficient solid particles to fill the interface, the tendency to contract would cause the interface to bend in the direction of the more poorly wetting liquid. Consequently the latter would become the internal phase. For a powder to remain at the interface, the angle of contact had to be finite; unless the angle was 90°, the interface was on one side or the other of the points of contact of the particles; and its tension caused the film to be concave on that side.

Clayton (38) stated that the angle of contact is that angle obtained when a solid meets the interface between two immiscible liquids and has profound importance for the theory of emulsions stabilized with insoluble solid particles.

If the solid (considered a sphere) meets the interface with the latter in line with its own equator, the angle of contact is exactly 90°. The solid would then exhibit preferential attraction for neither liquid. This, however, is practically a hypothetical case.

When a solid is shaken in a two-phase system, such as oil and water, the following cases must be considered with respect to interfacial tension:

(*a*) If $\sigma_{s,o} > \sigma_{s,w} + \sigma_{w,o}$ the solid will be completely immersed in the water phase

(*b*) If $\sigma_{s,w} > \sigma_{s,o} + \sigma_{s,o}$ the solid will be completely immersed in the oil phase

(*c*) If $\sigma_{w,o} > \sigma_{s,o} - \sigma_{s,w}$ the solid will be distributed in the interface

where:

$\sigma_{s,o}$ = interfacial tension between solid and oil
$\sigma_{s,w}$ = interfacial tension between solid and water
$\sigma_{w,o}$ = interfacial tension between water and oil

Since one of the liquids will exhibit preferential wetting of the solid, the equation for equilibrium becomes:

$$\sigma_{s,o} - \sigma_{s,w} = \sigma_{w,o} \times \cos\theta$$

If $\sigma_{s,w}$ is less than $\sigma_{s,o}$ then $\cos\theta$ is positive and θ is less than 90°. The greater portion of the solid is then surrounded by water so that its equator lies in the water phase. Similarly, if $\sigma_{s,o}$ is less than $\sigma_{s,w}$ then $\cos\theta$ is negative and θ is greater than 90°, the major portion of the solid being surrounded by the oil phase.

Other contributions relative to the mechanism of emulsification with insoluble solids have been reported (39–46).

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† Present address: North Carolina Pharmaceutical Research Foundation Fellow.

Although the ability to promote emulsification is possessed by a number of insoluble compounds, many investigators, for example Bennister and his associates (25), considered that the use of insoluble solids as emulsifiers generally was limited because the emulsions formed were coarse in quality. However, the investigators believed that there were many features of solid-stabilized emulsions which were sufficiently attractive to stimulate further research. In particular, they listed low cost and the fact that solids appeared to be so little affected even in the presence of strongly reactive electrolytes.

The present investigation was undertaken because there are many instances in extemporaneous compounding when emulsification with an insoluble solid could improve the appearance and effectiveness of the prescribed combination. Too, the literature has revealed little quantitative data with respect to pharmaceutical emulsions of this type.

EXPERIMENTAL

Preliminary.—Preliminary studies with fresh and aged magmas of magnesium carbonate showed that emulsion quality decreased with aging of the magma. On this basis and the fact that Bennister, *et al.*, had previously shown that the quality of emulsions prepared with aged magmas varied with the solid employed, the magmas were prepared immediately prior to use.

A study designed to determine the effect of particle size on emulsion quality revealed that particle size prior to homogenization was of secondary importance, since the process effected a reduction in the size of the solid particles.

It was found that optimum quality was obtained when the emulsifier was wetted with the aqueous phase followed by the addition of oil.

Preliminary studies also revealed that differences in quality existed in emulsions prepared from identical concentrations of ingredients whether passed through the same or different homogenizers. To effect greater uniformity, 3 emulsions were prepared for each concentration of emulsifier and the average of the data reported. The same homogenizer was used throughout the study and the tension on the spring at the aperture, once set, was not changed.

The temperature of distilled water and liquid petrolatum was adjusted to 25° prior to use.

Method of Preparation.—The oil phase of the emulsions was represented by 40% v/v liquid petrolatum. Emulsifier concentrations were 5% w/v, 3% w/v, and 1% w/v except for magnesium oxide. The latter exception was made because 5% w/v proved excessive and an almost solid product was obtained. Solids were oven dried at 105° for 48 hours. When such a temperature was contraindicated, the solid was dried over sulfuric acid in a desiccator for 48 hours.

To the solid emulsifier was added sufficient distilled water to make 135 cc. of magma. To the magma was added 90 cc. of liquid petrolatum which contained 0.1% Sudan II. After vigorously shaking 50 times, the mixture was passed through a hand homogenizer 4 times. All of the emulsions prepared in this study were of the oil-in-water type.

Screening Test.—A screening test was devised to select the more promising emulsifiers. Evaluation was based on centrifuge data obtained when samples of emulsions were centrifuged at 1,450 r. p. m. for one hour. This method was chosen because it afforded a quick and reproducible means of evaluation (47)

Five per cent concentration of the emulsifier was used except in the case of magnesium oxide.

The following insoluble solids were selected for the screening test: Barium sulfate, U. S. P. (Baker and Adamson); bismuth subcarbonate, U. S. P. (Baker and Adamson); bismuth subgallate, N. F. (Merck); bismuth subnitrate, N. F. (Merck); bismuth subsalicylate, U. S. P. (Merck); calcium carbonate, U. S. P. (Merck); calcium phosphate, dibasic (Fisher); calcium phosphate, tribasic, N. F. (Baker); cerium oxalate, N. F. (Merck); ferric oxide (Coleman and Bell); infusorial earth (Fisher); kaolin, N. F. (Merck); magnesium carbonate, U. S. P. (Baker and Adamson); magnesium hydroxide (Fisher); magnesium oxide, U. S. P. (Baker); magnesium trisilicate, U. S. P. (Baker); sulfur, precipitated, U. S. P. (Baker); talc, U. S. P. (Mallinckrodt); titanium dioxide (Baker and Adamson); zinc oxide, U. S. P. (Merck); zinc stearate, U. S. P. (Baker).

Results of the test indicated that magnesium oxide, magnesium hydroxide, tribasic calcium phosphate, kaolin, ferric oxide, zinc oxide, and magnesium carbonate were the best of the agents evaluated. Forty per cent liquid petrolatum emulsions stabilized with these solids were then subjected to a comprehensive investigation.

Talc, magnesium trisilicate, bismuth subsalicylate, cerium oxalate, infusorial earth, dibasic calcium phosphate, zinc stearate, titanium dioxide, and precipitated sulfur failed as emulsifiers.

DATA

Data were collected on the fresh emulsions, emulsions aged 7 days, emulsions aged 14 days, and emulsions aged 28 days. Between time intervals, the emulsions were stored at room temperature in amber, screw cap, paraffin-sealed bottles. Data were determined at 25°.

Mean Globule Diameter and Interfacial Area.—On the basis of the success achieved by Smith and Grinling (48), Cockton and Wynn (49), and Levius and Drommond (50), the method of globule count was used to determine the mean globule diameter and the interfacial area.

The method was modified somewhat. The counting chamber employed in this study was of the Fuchs-Rosenthal variety certified by the National Bureau of Standards. The data collected in preliminary experiments indicated that a cell depth of 0.2 mm. was preferable to the 0.1 mm. depth. The deeper cell obviated distortion and allowed dispersed globules as large as 200 μ to enter unhindered. The cell also afforded full view of the ruled area when the globules were in focus. The chamber consisted of 16 squares, each 1 sq. mm. in area, orientated by triple lines; and each square was subdivided into 16 smaller squares. Five of the larger squares constituted the count area and represented 1 cu. mm.

TABLE I.—MEAN GLOBULE DIAMETER OF THE DISPERSED PHASE OF 40% LIQUID PETROLATUM EMULSIONS STABILIZED WITH SELECTED SOLIDS

Concn., %	Age of Emulsion, Days	Mean Globule Diameter of the Dispersed Phase, μ						
		Mag. Hydrox.	Mag. Oxide	Kaolin	Ca. Phos. 3°	Ferric Oxide	Zinc Oxide	Mag. Carb.
5	Fresh	9.62	...	30.52	32.98	35.11	47.77	52.52
	7	9.65	...	30.78	33.59	36.92	49.97	55.30
	14	9.78	...	31.01	35.18	38.08	50.62	57.16
	28	10.01	...	32.02	36.31	39.55	53.74	59.34
3	Fresh	13.52	11.83	42.30	33.32	47.05	86.49	72.53
	7	13.63	11.90	44.20	47.93	50.62	87.22	73.56
	14	13.88	11.96	44.41	48.28	53.51	80.75	73.96
	28	13.97	12.01	44.74	49.71	54.83	81.71	78.40
1	Fresh	28.24	20.47	94.32	51.98	73.61	Unsatisfactory	
	7	28.70	20.75	98.75	65.24	101.25	...	...
	14	29.31	20.81	97.05	70.55	94.32	...	...
	28	29.47	20.83	102.93	76.14	106.28	...	...

of volume. The five count areas were located in the four respective corners of the ruled chamber and the fifth near the center.

The emulsion was diluted to the appropriate volume and the cell charged with the diluted emulsion after the cover slip had been placed in position. This operation was conducted as quickly as possible to avoid uneven distribution of globules in the chamber. Once charged, the cell was allowed to stand 10 minutes to permit the dispersed globules to rise to the surface. A preliminary examination of the slide was made to insure even distribution of the droplets. When poor distribution was observed, the slide was cleaned and refilled. Dilution of the emulsions varied from 1–50 through 1–1,000. Duplicate counts were made on each of 3 emulsions and the average of the six counts were used in the calculations.

The dilution medium consisted of 25% acacia mucilage and was chosen after a preliminary test was conducted which indicated that the substance was the best investigated on the basis of compatibility and suspending power. Other compounds considered were glycerin, 50% aqueous glycerin, propylene glycol, 50% aqueous propylene glycol, 2% carboxymethylcellulose, 5% carboxymethylcellulose, 1% aerosol O. T., and distilled water. To avoid accidental emulsification, the dilutions were never subjected to agitation. The dilution medium and emulsion were always adjusted to 25° prior to mixing.

To avoid counting globules twice, the count for each square included all globules which lay on or touched the top and left side of the square and excluded all those which lay on or touched the bottom and right side. After each count, the slide was washed with soap solution, rinsed with tap water, distilled water and then dried with a soft cloth.

Calculations which involved data obtained from globule count were made as follows:

(*a*) The volume under 80 small squares was calculated to be: 80(0.2 mm. × 0.25 mm. × 0.25 mm.) = 1 mm.^{3} (*b*) The number of globules into which 1 mm.^{3} of oil was subdivided was calculated from the formula: $H = N \times 1 \text{ mm.}^3 \times Z \times (100/40)$ where: H = number of globules into which 1 mm.^{3} of oil was subdivided, N = number of globules counted in 80 squares and Z = dilution factor.

(*c*) The mean globule diameter was calculated from the formula: $V_g = 4/3\ \pi r^3$ or $\pi D^3/6$; 1 mm.^{3} = $(\pi D^3/6) \times H$; $D^3 = (6 \times 1 \text{ mm.}^3)/(\pi \times H)$; $D_{mm} = \sqrt[3]{(6 \times 1 \text{ mm.}^3)/(\pi \times H)}$; where: D_{mm} = diameter in mm., and V_g = volume of a globule (assumed spherical). (*d*) The interfacial surface given by 1 cc. of oil was calculated: $S_g = \pi D\text{cm.}^2$; $S_t = \pi D\text{cm.}^2 \times H$; $S_i = \pi D\text{cm.}^2 \times H \times (1/10^{-2})$; $S_i = \pi D\text{cm.}^2 \times H \times 10^2$ where: S_g = surface area of 1 globule; S_i = total surface area in cm.^{2}/cc. of oil.

Viscosity.—Viscosity was determined with Ostwald-Fenske viscometers. Because of the rapid rate of creaming of many of the emulsions, it was

TABLE II.—INTERFACIAL AREA OF ONE CUBIC CENTIMETER OF LIQUID PETROLEUM EMULSIFIED WITH SELECTED SOLIDS

Concn., %	Age of Emulsion, Days	Interfacial Area, Cm.^{2}						
		Mag. Hydrox.	Mag. Oxide	Kaolin	Ca. Phos. 3°	Ferric Oxide	Zinc Oxide	Mag. Carb.
5	Fresh	6,250	...	1,964	1,818	1,708	1,255	1,141
	7	6,228	...	1,948	1,797	1,624	1,200	1,084
	14	6,140	...	1,934	1,704	1,575	1,184	1,049
	28	6,010	...	1,872	1,651	1,516	1,116	1,010
3	Fresh	4,434	5,069	1,417	1,799	1,274	693	827
	7	4,400	5,039	1,357	1,251	1,184	687	815
	14	4,320	5,015	1,350	1,242	1,121	742	810
	28	4,293	4,990	1,340	1,206	1,093	733	765
1	Fresh	2,123	2,930	636	1,153	815	Unsatisfactory	
	7	2,089	2,889	607	919	592	...	...
	14	2,045	2,881	618	850	635	...	...
	28	2,035	2,879	582	788	564	...	...

TABLE III.—VISCOSITY OF 40% LIQUID PETROLATUM EMULSIONS STABILIZED WITH SELECTED SOLIDS

Concn., %	Age of Emulsion, Days	Viscosity in Centipoises of 40% Liquid Petrolatum Emulsions Stabilized with— Mag. Oxide	Mag. Hydrox.	Ferric Oxide	Ca. Phos. 3°	Mag. Carb.	Kaolin	Zinc Oxide
5	Fresh	...	295.0	42.1	37.4	39.5	16.7	12.6
	7	...	139.5	45.0	45.9	38.9	18.2	13.4
	14	...	118.9	43.6	42.5	37.3	16.8	12.6
	28	...	100.6	41.1	34.1	39.5	15.9	11.9
3	Fresh	...[a]	27.1	12.6	11.2	6.6	8.2	7.0
	7	...[a]	22.5	12.2	9.3	6.9	8.1	6.7
	14	959.1	20.7	12.0	8.8	7.2	8.2	6.4
	28	612.5	18.8	11.8	8.2	7.0	7.7	6.2
1	Fresh	30.4	8.5	4.9	4.3	Unsatisfactory	4.2	Unsatisfactory
	7	21.0	8.5	4.6	4.3	...	4.1	...
	14	13.3	7.8	4.3	4.3	...	3.8	...
	28	10.4	7.5	3.9	3.8	...	3.7	...

[a] Viscosity too great to be determined.

necessary to swirl the charged viscometers gently for 10 seconds immediately prior to determining efflux time. The procedure was repeated five times and the average was used to calculate viscosity.

pH.—*p*H values were determined with a Model N, Beckman *p*H meter. In some cases emulsions caused erratic responses of the meter. This was especially true with emulsions stabilized with tribasic calcium phosphate and zinc oxide.

Centrifugation.—The separation of the internal phase was determined by utilizing the acceleration obtained by the use of centrifugal force. The centrifuge employed was a Size 1, Type C, International centrifuge equipped with a horizontal head for 8 fifty-cc. tubes and was operated at a speed of 1,450 r. p. m.

Two 25-cc. samples from each of 3 emulsions prepared for each concentration of emulsifier were measured into heavy duty conical bottom tubes and centrifuged for one hour. To prevent evaporation, the tubes were covered with thin rubber diaphragms. The average was reported.

DISCUSSION

Screening Test.—Several interesting facts were observed during the screening test. Bismuth subsalicylate, zinc stearate, and precipitated sulfur exhibited their well-known aversion for water and consequently failed as emulsifiers. However, a small amount of glycerin permitted the wetting of the subsalicylate and stearate and acceptable emulsions were produced. In the case of precipitated sulfur, a small amount of aerosol, O. T. functioned as did the glycerin in permitting the wetting of the solid by water. These emulsions were not investigated further.

Mean Globule Diameter and Interfacial Area.—A rather rapid rise of the globules was noted in dilutions prepared even in so viscous a medium as 25% acacia mucilage. This was caused by the relatively large diameters of the dispersed phase. It was anticipated, therefore, that precise measurements would be difficult and an average of two globule counts for each of 3 emulsions prepared for each concentration of emulsifier would better serve for purposes of calculation. In a few cases, erratic results were obtained in which the later count slightly exceeded the count recorded for a previous determination. When such data were obtained, they were included in the calculation for the average. In the opinion of the authors, such inconsistency is inherent in the sampling procedure due to the nature of the emulsions investigated. Greater precision is impractical.

The mean globule diameter of the dispersed phase decreased inversely with the concentration of the emulsifier and increased with aging of the emulsions.

The interfacial area decreased with concentration of the emulsifier and with aging of the emulsions.

Viscosity.—With 3% and 1% concentrations of emulsifier, viscosity decreased with aging of the emulsion. However, with 5% concentration, viscosity exhibited somewhat erratic behavior in that a

TABLE IV.—*p*H OF 40% LIQUID PETROLATUM EMULSIONS STABILIZED WITH SELECTED SOLIDS

Concn., %	Age of Emulsion, Days	*p*H of Emulsions Stabilized with— Mag. Oxide	Mag. Carb.	Mag. Hydrox.	Zinc Oxide	Ca. Phos. 3°	Ferric Oxide	Kaolin
5	Fresh	..	10.3	10.0	8.0	7.9	6.6	4.9
	7	..	10.1	9.9	8.0	7.8	6.5	5.1
	14	..	10.0	9.9	8.0	7.8	6.5	5.1
	28	..	9.9	9.9	8.2	7.8	6.5	5.1
3	Fresh	10.8	10.3	10.0	8.0	7.6	6.7	5.0
	7	10.6	10.0	9.9	8.0	7.6	6.6	5.3
	14	10.5	10.1	9.9	8.0	7.5	6.6	5.3
	28	10.3	10.0	9.9	7.7	7.6	6.7	5.3
1	Fresh	10.7	Unsatisfactory	10.0	Unsatisfactory	7.7	6.3	5.6
	7	10.6	..	9.9	..	7.5	6.2	5.8
	14	10.6	..	9.9	..	7.5	6.2	6.0
	28	10.6	..	10.0	..	7.5	6.2	6.0

TABLE V.—OIL SEPARATED FROM 40% LIQUID PETROLATUM EMULSIONS STABILIZED WITH SELECTED SOLID AFTER CENTRIFUGING AT 1450 R. P. M. FOR ONE HOUR

Concn., %	Age of Emulsion, Days	Oil in Cc. Separated from Emulsions Stabilized with: Mag. Hydrox.	Mag. Oxide	Zinc Oxide	Ferric Oxide	Kaolin	Mag. Carb.	Ca. Phos. 3°
5	Fresh	0[a]	..	T	T	0.2	0.6	0.9
	7	0	..	0.2	T	0.2	0.6	0.7
	14	0	..	0.2	T	0.2	0.6	0.3
	28	0	..	0.4	T	0.2	0.6	0.4
3	Fresh	T[b]	0	3.0	0.2	0.2	6.1	4.9
	7	0	0	3.0	0.2	0.2	6.1	5.1
	14	0	0	6.1	0.2	0.2	6.8	2.8
	28	0	0	6.1	0.2	0.3	6.7	2.6
1	Fresh	0.1	2.1	Unsatisfactory	7.3	5.9	Unsatisfactory	9.2
	7	0.1	1.0	..	7.5	4.0	..	9.3
	14	0.1	1.4	..	7.1	3.6	..	9.3
	28	0.1	1.3	..	6.7	3.9	..	9.0

[a] O = Complete absence of oil.
[b] T = Trace.

slight increase was recorded after seven days, followed by a decrease. Such a sequence was shown by emulsions stabilized with tribasic calcium phosphate, ferric oxide, and kaolin. A possible explanation for this action could be a slow rate of hydration for the solids, which after reaching a maximum, show a decrease; or the viscosity was later reduced by other changes in the systems.

pH.—The *p*H data have little significance in evaluating quality. The hydrogen ion concentration was dependent upon the solid used as the emulsifier and there was no consistent influence of *p*H on emulsion quality when comparing different emulsifiers.

It is interesting pharmaceutically that oxides and hydroxides give liquid petrolatum emulsions of *p*H 6.5–10.8 (ferric oxide to magnesium oxide); tribasic calcium phosphate, *p*H about 7.7; and kaolin, *p*H about 5.5.

Centrifugation.—Data pertaining to oil separated by centrifuging revealed, for the most part, changes of little significance. In a few cases, it was noted that a decrease of oil was released upon centrifuging the aged emulsions. Explanation for this change is difficult. It is possible to suggest, however, that such changes involved the interfacial film whose strength varied with emulsifier and concentration and with the time required to become stabilized.

SUMMARY

1. Seven solids were evaluated as emulsifiers for 40 per cent v/v liquid petrolatum, U. S. P. On the basis of mean globule diameter, they could be rated in order of decreasing efficiency, at the 3 per cent concentration level, with magnesium oxide giving a mean globule size of 11.83 μ through magnesium hydroxide, tribasic calcium phosphate, ferric oxide, and magnesium carbonate, to zinc oxide, mean globule diameter 86.49 μ.

2. Less efficient emulsifiers, eliminated in a screening test were: bismuth subgallate, bismuth subcarbonate, calcium carbonate, barium sulfate, and bismuth subnitrate. Solids, which failed as emulsifiers in the screening test, were: talc, magnesium trisilicate, bismuth subsalicylate, cerium oxalate, infusorial earth, dibasic calcium phosphate, zinc stearate, titanium dioxide, and precipitated sulfur.

3. Though the mean globule diameters of solid-stabilized emulsions did not compare favorably with those of standard, soluble emulsifiers, satisfactory emulsions with relatively low viscosities were produced.

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Investigation of the Toxic Principles of *Hippomane mancinella* L. III.*

Toxic Actions of Extracts of *Hippomane mancinella* L.

By MARCUS N. CARROLL, JR.,† LAURETTA E. FOX,‡ and WILLIAM T. ARIAIL

Manchineel fruits or extracts of fruits, leaves or wood of manchineel, when fed or injected into animals produced extensive hemorrhages. Toxic principles were soluble in water, alcohol, or oil. The blood pressure fall was antidoted with ephedrine and the salivation and lacrimation with atropine.

THE HISTORICAL LITERATURE dealing with *Hippomane mancinella* L. was reviewed by Lauter, Fox, and Ariail (1). Schaeffer, Lauter, and Foote (2) reported the extraction of brevifolin by steam distillation of the leaves and of α-carotene from the alcoholic extract of the leaves. Lauter and Foote (3) obtained a principle from the fruits which showed a remarkable resemblance to physostigmine. Harley (4) states that the leaves and branches when broken extrude a milk latex which is extremely irritating to the mucous membranes and skin.

The toxic principles are referred by most workers to the latex (5–7), although Earle (6) states that the dried wood and sawdust have irritant properties and raindrops falling off the trees or in stagnant pools may have a suspension or solution of the toxic ingredients. Coutance, according to Earle, found a very hard greenish resin, insoluble in ether or alcohol which was the active principle and an inactive resin which was soluble in ether and alcohol, as well as an inactive essential oil. Malowan (8) extracted a vegetable resin which produced the same symptoms in animals as those produced by the latex.

The present study was undertaken to screen the extracts for the noxious effects reported in legends, historical literature, and medical reports and to record any toxic symptoms hitherto unknown. It was hoped that some antidote might be found.

EXPERIMENTAL

Acute Toxicity.—Greenish colored fruits of *Hippomane mancinella* L. picked from trees in the Everglades National Park, Homestead, Fla., in August, 1951, were offered *ad libitum* as the sole diet for five days to 20 albino rats that had been fasted forty-eight hours prior to the experiment. The rats ate 1 or 2 fruits but no more even though no other food was available. The quantity of fruits consumed did not produce obvious symptoms of poisoning. The rats ate heartily when returned to a commercial ration.

The whole green fruits were ground and passed through a 60-mesh screen and force fed to rabbits

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† Present address: Miles-Ames Research Laboratory, Elkhart, Ind.
‡ Associate Professor of Pharmacognosy and Pharmacology, College of Pharmacy, University of Florida, Gainesville.
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and rats. Four groups of 5 rats survived doses of 0.10, 1.0, 5.0, and 11.0 Gm. of fresh fruit per Kg. of body weight without showing any signs of poisoning. Three groups of 3 rabbits each survived doses of 5.0, 15.0, and 25.0 Gm. of fresh fruit per Kg. of body weight without showing obvious signs of poisoning. The average weight of the fruits of this collection was 5.7 Gm.

Aqueous and alcoholic extracts, and the residue of the alcoholic extract prepared by Lauter and Foote (as described in the section of their article, "Preliminary alcoholic extraction") (3), from fruits collected in July, 1950, produced toxic symptoms in guinea pigs. Guinea pigs injected with the residue manifest lacrimation, depression of respiration, skeletal muscle fibrillation, clonic then tonic convulsions, prostration, and death. A 100% fatal dose was 0.02 Gm. of the residue of the alcoholic extract (equivalent to 0.8 Gm. of fresh fruit) per Kg. of body weight. The average weight of the fruits in this collection was 4.7 Gm.

Some fruits of the July, 1950, collection were stored at room temperatures from July, 1950, to October, 1956. They were cut, ground, and extracted with sesame oil, 20 Gm. of fruit to 30 ml. of oil.

A group of albino rats, weighing about 100 Gm. each, was injected intraperitoneally with 20 ml. of the oil extract per Kg. of body weight and a group of controls with the same amount of sesame oil. All 7 rats injected with the sesame oil extract expired within seventy-two hours. The controls remained alive and asymptomatic.

A group of albino rats given 10 ml. of the sesame oil extract per Kg. suffered from symptoms of poisoning and 5 of the 8 succumbed in seventy-two hours, while 7 of the 8 receiving 5 ml. per Kg. survived without showing obvious signs of poisoning.

Aqueous and alcoholic extracts of both wood and leaves prepared by Schaeffer, Lauter, and Foote (2) when fed or injected into mice, rats, guinea pigs, or rabbits caused lacrimation, salivation, acceleration of respiratory rate, dyspnea, marked fall in blood pressure [using indirect method of Voss and Johnson (9)], depression (crawled to a corner of the cage and remained inactive even when tail was pinched with forceps), slowing of heart rate, diarrhea, skeletal muscle twitching, clonic then tonic convulsions, then slow forced respiration until death from respiratory paralysis or recovery ensued. Post-mortem examination revealed a fibrillating heart, apparent dilation of the right side of the heart, and petechial hemorrhages which stained the viscera and fat pink or red in all species except the guinea pig. In the latter species there were massive hemorrhages in the viscera and fatty tissues.

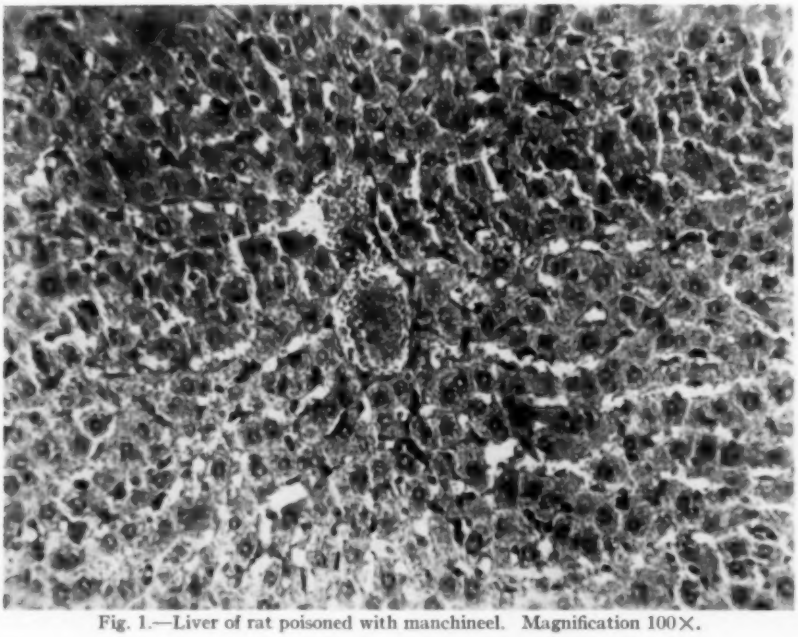

Fig. 1.—Liver of rat poisoned with manchineel. Magnification 100×.

A sesame oil extract of finely shredded manchineel wood, 0.25 Gm. of shredded wood per ml. of oil, produced the same type of symptoms when fed or injected into albino rats or other species of animals; for rats the minimum fatal dose of the oil extract was 30 ml. (representing 7.5 Gm. of wood)/Kg. of body weight.

Slides prepared from tissues of the ileum, heart, spleen, liver, and kidney of the acutely-poisoned rats and stained with hematoxylin and eosin showed no changes in the tissues of the ileum, heart, or spleen. The sinusoids of the liver were congested and hemor-

rhagic foci were seen throughout the sections (Fig. 1). The kidneys had generalized areas of hemorrhage, expecially the areas of the collecting tubules (Fig. 2).

A concentrated aqueous extract of wood prepared by Schaeffer, Lauter, and Foote (2) and referred to by these authors as "concentrated extract" was administered to groups of 10 albino rats. One dose of 0.2 ml. or 0.1 ml. of this extract per Kg. of body weight proved 100% fatal, while one dose of 0.05 ml. per Kg. was rarely fatal.

Slides prepared from the tissues of albino rats surviving the latter dose showed that no histological changes were evident two weeks after the extract was administered. One week after the extract was administered no gross changes were observed but microscopic examination of the same tissues showed hemosiderin deposits in the cells of the spleen and several months manifest the same type of symptoms and tissue pathology as those poisoned acutely.

Toxalbumin.—It was suggested that the extracts might contain a toxalbumin. Extracts of the wood prepared and tested according to the method of Bamford (10) yielded negative results.

Histamine.—Using the procedure of Friedman (11) no changes in free or total hydrochloric acid of the stomachs of cats could be determined after administering the concentrated aqueous extract.

Blood Changes.—Since the odor of the extracts suggested coumarin it was suggested that blood changes be studied. There were no significant changes in coagulation time or bleeding time (12) of albino rats after administration of the concentrated aqueous extract. Rabbits did not show any change in prothrombin time (13, 14). Using the method of

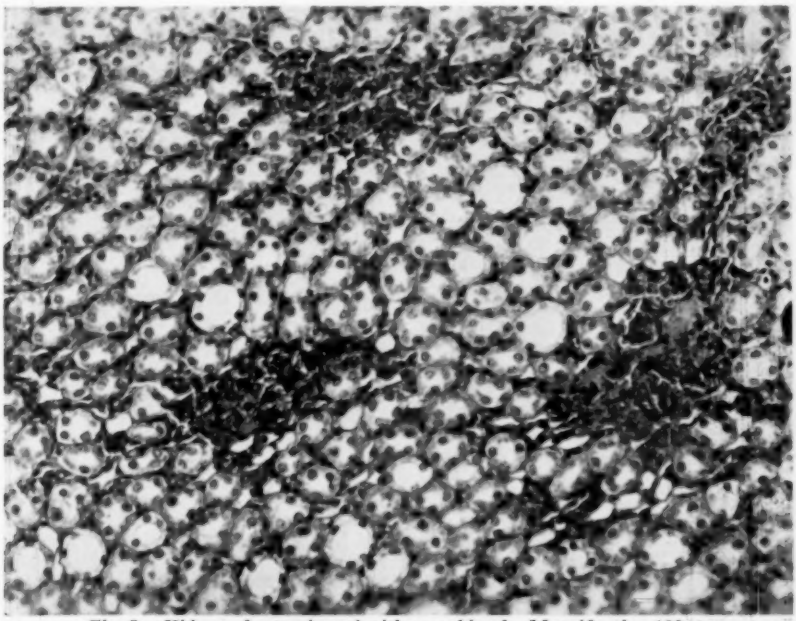

Fig. 2.—Kidney of rat poisoned with manchineel. Magnification 100×.

areas of hemorrhage, cloudy swelling and parenchymatous degeneration in the liver.

Guinea pigs frequently had extensive hemorrhagic areas in the stomach and intestine after receiving the extracts orally. The ovaries, pancreas, adrenal glands, liver, and kidneys were hemorrhagic. One guinea pig carrying young expired after oral administration of the extracts. The feti were hemorrhagic and had died some time before their dam. Guinea pigs lived from three to sixteen days after administering the extracts, unlike the rats they showed areas of extensive hemorrhage in the viscera and fat. Mice and rabbits manifest the same type of hemorrhage as the rats.

Chronic Toxicity.—Guinea pigs and albino rats receiving 0.05 ml. of concentrated aqueous extract per Kg. of body weight twice a week over a period of

Bamford (15) the extracts did not hemolyze red blood cells. Albino rats treated with nonfatal doses of the extracts did not show significant changes in hemoglobin content or red blood cell count (16). However two weeks after receiving the first dose of the extract the rats showed a marked leukocytosis (17) without changes in other cellular elements (Table I).

Blood Pressure.—Intravenous administration of concentrated aqueous extracts of the wood suspended in 0.9% saline (2) to dogs under pentobarbital anesthesia and prepared to record blood pressure directly, showed an abrupt fall in blood pressure immediately following administration of the extracts (the average drop in six dogs being 75 mm. Hg). Epinephrine hydrochloride (1 ml. of 1:10,000) or ephedrine sulfate (1 ml. of 1%) counteracted

TABLE I.—RESULTS OF DIFFERENTIAL COUNTS ON ALBINO RATS TREATED WITH MACHINEEL EXTRACT

	Control			Experimental		
	Range, %	Mean, %	S. D.	Range, %	Mean, %	S. D.
Lymphocytes	83–98	91.5	5.5	63–92	84.0	9.7
Segmented polys.	2–11	5.8	4.0	3–25	10.5	7.7
Band polymorphs.	1–4	1.2	1.0	1–8	3.3	2.2
Eosinophils	0–1	0.1	0.0	0–3	0.3	0.5
Monocytes	1–3	1.4	1.4	1–3	1.6	1.1

T values: Lymphs. (2.1), segs (1.7), bands (27.0), eosins. (0.2), monos. (0.2).

this fall in blood pressure. If untreated, the blood pressure remained indefinitely at about 60 mm. Hg.

Irritation in Animals.—No skin irritation was produced in animals by methods used (18).

Instillation of the concentrated aqueous extract of the wood (buffered to *p*H 7.0 with Gifford's buffer) into the eyes of rabbits produced chemosis, severe conjunctivitis, and blephrospasm. However, shaking the extract with cholesterol before instilling it rendered it innoxious. Figure 3 shows the typical response thirty minutes after treatment with the buffered extract and Fig. 4 shows the action of a portion of the extract mixed with cholesterol. On the following day the eyes treated with the buffered extract were filled with a muco-purulent discharge. Instillation of a 2% sodium fluorescein solution (4) failed to reveal denuded corneal epithelium. However, in one of every five rabbits used, clouding of the cornea was produced and persisted.

Irritation in Workers.—During the course of grinding the manchineel wood for preparation of the extracts one of the workers was forced to breathe in the area where dusts were given off and did not use a respirator. One hour after the grinding procedure he suffered from severe burning in the nasal passages and a dull persistent headache. He was treated in the University Infirmary (19) with warm saline irrigation of the nasal passages followed by a nasal spray containing epinephrine and tetracaine. He was free of symptoms in six hours.

Fig. 3.—Rabbit eye thirty minutes after instillation of extract of manchineel.

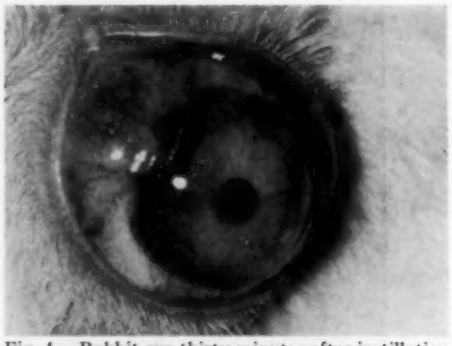

Fig. 4.—Rabbit eye thirty minutes after instillation of cholesterol-treated extract of manchineel.

TABLE II.—IRRITATION OF RABBIT'S EYE WITH AQUEOUS EXTRACT OF WOOD

Time After Distillation	Maximum Points	Points After 30 Min.	6 Hr.	24 Hr.	48 Hr.
I. Cornea					
a. Opacity	4	0[a]	0.8	0.8	0.8[b]
b. Area Involved	4	0[a]	0.8	0.8	0.8[b]
(Score equals $a \times b \times 5$)					
II. Iris					
a. Values	2	0	0	0	0
(Score equals $a \times 5$)					
III. Conjunctivae					
a. Redness	3	3	3	0	0
b. Chemosis	4	4	4	1	0
c. Discharge	3	1	3	3	1
Score equals $(a + b + c) \times 2$					
Score	110	16	52	40	34

[a] Average for 5 rabbits.
[b] When positive, complete and permanent opacity but not frequently observed.

During the course of cutting and grinding the fruits stored from July, 1950, to October, 1956, one of the authors did not use a respirator and inhaled the dusts which caused, some forty-five minutes after the procedure was started, severe burning in the nasal passages, a dull headache and nausea. No treatment was instigated. After six hours the author was symptom free.

Antidotes.—Groups of albino rats given 0.1 ml. of concentrated aqueous extract (2) per Kg. of body weight and antidoted with caffeine and sodium benzoate (100 mg./Kg.), nikethamide (150 mg./Kg.) pentylenetetrazol (30 mg./Kg.), or ephedrine hydrochloride (100 mg./Kg.) lived an average of 115, 135, 125, and 105 minutes, respectively, as compared to those antidoted with procaine hydrochloride (100 mg./Kg.), cocaine hydrochloride (20 mg./Kg.), or neostigmine methylsulfate (0.5 mg./Kg.) and diphenhydramine hydrochloride (15 mg./Kg.) that survived 74, 81, 27, and 39 minutes, respectively; the controls surviving an average of 43 minutes. Atropine sulfate (350 mg./Kg.) overcame the symptoms of lacrimation and salivation and the group survived an average of 107 minutes. Administration of vitamin C (100 mg./Kg./day) or hesperidin methylchalcone (100 mg./Kg./day) for two weeks before treatment with the extracts did not prevent the petechial hemorrhages nor the fall in blood pressure in albino rats or rabbits [blood pressure by method of Johnson and Voss (9)].

DISCUSSION

Various authors record considerable variance in the toxicity of the fruits, leaves, and wood of the manchineel tree (20–22). For example, one man suffered from tasting a fruit (23) while another ate 24 fruits without being poisoned (24) and a pregnant woman longed for the fruits and ate them without ill effect (20).

These differences have been attributed to seasonal variations, geographic location, amount of latex present in the plants, and other factors (22). There was considerable variation in the toxicity of the fruits collected in July, 1950, and in August, 1951. The latter small green fruits were pulled from the trees, while those collected in 1950 were ripe, yellow "apples" that were picked from the ground.

There are considerable references to the poisonous substances vaporizing on storage (5, 25). Fruits stored from July, 1950, to October, 1956, proved very toxic to both the author and animals.

Animals poisoned acutely with extracts of manchineel presented symptoms not unlike the syndrome reported in poisoned individuals (21, 26–30). There is no previous record of internal hemorrhage; however, the medical reports record shock or a collapsed condition (6, 26, 28).

The skin irritations recorded in the literature (6, 21, 30, 31, 32) were not reproducible in animals. The irritation in rabbit's eyes resembled the cases of irritation of the eyes of poisoned individuals (4, 6). The blood findings in animals paralleled those recorded for poisoned individuals, where leukocytosis is the only significant finding (6).

One of the earliest suggestions on antidotes was to swallow oil (23). There are two records of poisoning from extraction of manchineel in fats (20, 21). After ingestion of manchineel, lavage with oil followed by a saline cathartic [instead of a purgative (33)] is recommended for treatment.

The fall in blood pressure can be antidoted in animals with epinephrine or ephedrine. The salivation and lacrimation can be counteracted with atropine.

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Rauwolfia Hypotension I.*

The Role of the Vagi and Carotid Afferent Nerves

By CARL A. SCHLAGEL† and JOHN W. NELSON‡

Four groups of dogs, each with surgically different backgrounds were given 1.0 mg./Kg. of alseroxylon alkaloids intravenously and observed for six hours for changes in blood pressure, heart rate, and carotid occlusion reflex responses. The same degree of bradycardia was observed in each of the four groups of animals. However, the blood pressure fell to a lower level in the completely denervated dogs than in the normal, vagotomized, or carotid denervated animals. It is therefore evident that neither the vagi nor afferent nerves at the carotid bifurcation is necessary for mediation of the bradycrotic response. The alseroxylon-induced hypotension is also not mediated via these nerves, but is, instead, slightly limited or buffered by the presence of either or both of these nervous pathways. Whether this buffering action is physiological or potentiated by the alseroxylon alkaloids is reported upon elsewhere in This Journal.

THE PHARMACOLOGY of the alkaloids of *Rauwolfia serpentina* has been extensively studied during the past several years. Nevertheless, the precise mode of action of these alkaloids either singly or collectively is not yet completely understood.

Their general pharmacology depicts a syndrome of effects which implicates the central nervous system as their chief locus of action. Accordingly, many of the earlier investigators indicated that the typical pharmacological and clinical signs induced by the majority of the Rauwolfia alkaloids, such as hypotension, bradycardia, and tranquilization, were due to a direct reduction in activity of some central sympathetic regulating centers, such as the hypothalamus (1–3).

More recent studies, often using larger doses of the alkaloid(s), have suggested that the Rauwolfia alkaloids might have a far more generalized influence on the central nervous system than was originally assumed. Indeed, one investigator, using reserpine, concluded that the central sympathetic depressant effect of reserpine seemed not to be due to a direct depression of central sympathetic structures, but rather to a blocking or inhibition of afferent impulses which impinge upon and thereby activate these centers under normal conditions (4).

Two of the afferent nervous pathways which impinge upon the central nervous system and which are normally involved in regulating the mean arterial blood pressure are the afferent vagi and the afferent nerves at the carotid bifurcation. Pressor responses which normally occur upon proper stimulation of both of these nervous pathways are blocked due to the action of all of the commonly employed Rauwolfia preparations (2, 3, 5, 6). The ability of Rauwolfia to block these two nervous pathways was the particular pharmacological action which prompted the present study and dictated the direction of the investigation. Further impetus for the present study ensued from the following facts.

(*a*) The vagi and carotid nervous mechanisms normally assume an important buffering action on the arterial blood pressure.

(*b*) While it is generally accepted that Rauwolfia preparations show their greatest efficacy in the "so-called" essential hypertensive patient, one of their possibly manifold sites of action could be on the vagal and/or the carotid receptor nerve endings, since it has recently been postulated that a change in the intrinsic tone and resistance to stretch of the arterial walls where the sino-aortic pressoreceptors are located could be the primary mechanism of essential hypertension (7).

(*c*) The majority of the veratrum alkaloids or alkaloidal preparations given intravenously produce hypotension and bradycardia just as intravenous Rauwolfia produces hypotension concommitantly with bradycardia. The veratrum preparations have also been shown to produce their characteristic response when injected into the carotid sinus walls, probably by lowering the threshold of the "firing off" of the local receptor nerve endings (8). With larger doses of veratrum given intravenously, the carotid occlusion pressor reflex is also blocked just as it is following intravenous Rauwolfia preparations (9) Accordingly it would be interesting to learn if Rauwolfia

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Presented to the Scientific Section, A. PH. A., Detroit meeting, April, 1956.
† Fellow of The American Foundation for Pharmaceutical Education. Present Address: Upjohn Company, Kalamazoo, Mich.
‡ Present address: College of Pharmacy, The Ohio State University, Columbus 10.

might act on the carotid pressoreceptors similarly to veratrum. Such an observation would not only contribute to our knowledge of the pharmacodynamics of Rauwolfia, but would also add or detract from the rationale for the use of Rauwolfia-veratrum preparations in combination therapy.

The present investigation can conveniently be divided into two major experiments. In the first, which is reported here, the general role which the vagi and the afferent nerve endings at the carotid bifurcation assume in the characteristically mild but prolonged hypotension elicited by Rauwolfia was determined. In the second experiment, the results of which are published elsewhere in THIS JOURNAL, the alseroxylon alkaloids, reserpine, and other specifically selected drugs were tested in dogs for their specific local effect on the carotid pressoreceptor and chemoreceptor nerve endings.

EXPERIMENTAL

Mongrel dogs of both sexes and various weights were divided into four groups with the number of animals in each group shown in parentheses: Normal animals (3), vagotomized dogs (5), carotid denervated animals (5), and carotid denervated plus vagotomized dogs (5). After a control period for recording heart rate, blood pressure, and carotid occlusion reflex responses, all the animals received 1.0 mg./Kg. of the alseroxylon alkaloids intravenously whereupon they were observed for at least six hours and in a few instances for longer periods of time.

The femoral arterial pressure was recorded with a mercury manometer and the epsilateral femoral vein was cannulated for injection of the drug. To eliminate clotting in the tubing leading to the manometer, 10 to 15 mg. of heparin was added to an 8½% solution of sodium citrate in the arterial cannula and tubing. The respiration was recorded via a tracheal cannula.

An estimate of the heart rate was made by both counting the oscillations in the mercury manometer and with a stethoscope to the chest. The average of three one-minute determinations was taken as the heart rate for that period.

Since many of the studies required ten to twelve hours from the time of administration of the anesthesia until the termination of the experiment, the anesthetic chosen after numerous trials was the triad: sodium pentothal 2½% aqueous solution, 20% aqueous sodium barbital, and a 5% solution of α-chloralose in a 1-1 mixture of polyethylene glycol 400 and distilled water. The doses administered were: sodium pentothal, 10–15 mg./Kg., i. v.; followed immediately by sodium barbital, 140 mg./Kg., intraperitoneally; and α-chloralose, 50 mg./Kg. intraperitoneally. With this combination of agents and doses, the carotid occlusion reflex was usually powerful, and only an occasional supplemental dose of anesthetic was necessary.

Fresh solutions of the alseroxylon alkaloids were prepared by dissolving 50 mg. of the alkaloids in 1.5 ml. of a 50% citric acid solution and then diluting with distilled water to 10 ml. The final concentration of the citric acid and the alseroxylon alkaloids was 0.75% and 5.0 mg./ml., respectively. The *p*H of the solution was 2.2.

The vagotomies, tracheal cannulations, and bilateral carotid artery occlusions were performed at the midcervical level of the neck. At least one inch of each vagus was removed to lessen the chance for afferent or efferent vagal stimulation during the carotid clamping procedure. In the animals in which the carotid sinus zones (pressoreceptors plus chemoreceptors) were denervated, separate bilateral incisions were made directly over the areas of the carotid bifurcations.

The carotid sinus zones were completely denervated either by dissection and sectioning of the freed intracarotid branch of the glossopharyngeal nerve or by dissection and sectioning of this nerve together with the occipital and/or aberrant vessels along which it runs.[1]

In two animals (not included in the above four groups) the carotid pressoreceptors were differentially denervated, leaving the chemoreceptors intact by sharp dissection of the pressoreceptor nerve fibers between the carotid body and sinus. The wall of the common carotid artery where it approached the bifurcation, as well as the internal carotid artery, were carefully pinched with small forceps in an effort to disrupt any straggling pressoreceptor nerve fibers. Great care was taken to protect the blood and nerve supply of the carotid body. Thoroughness of pressoreceptor denervation was determined by abolition of the carotid occlusion pressor reflex response, while the integrity of the chemoreceptors was verified by the hyperpnea resulting from the intravenous injection of a freshly prepared solution of NaCN at a dose of 0.5 or 1.0 mg./dog.

In the intact (normal) and vagotomized animals, the blocking effect of Rauwolfia on the carotid pressor response was determined by occluding the common carotid arteries for approximately 45 seconds during a control period and at various intervals, usually every hour or sometimes more frequently, after injection of the drug.

RESULTS

To eliminate the need for the inclusion of a large mass of figures from the protocols of the individual animal experiments, certain details of the results of the 18 dogs of this experiment have been tabulated and are shown in Tables I, II, and III. These tables show the effect of the alseroxylon alkaloids on the heart rate, blood pressure, and the carotid occlusion reflex, respectively. In addition, one typical kymograph tracing with a detailed legend is shown for each of the four groups of animals tested (Figs. 1–4). These tracings adequately document the findings, while at the same time, they exhibit the characteristic effects of the drug over the complete six-hour period.

[1] The writers are grateful to Dr. S. C. Wang, Professor of Physiology, Columbia University, College of Physicians and Surgeons, who graciously supplied information relative to the method used for completely denervating the carotid area.

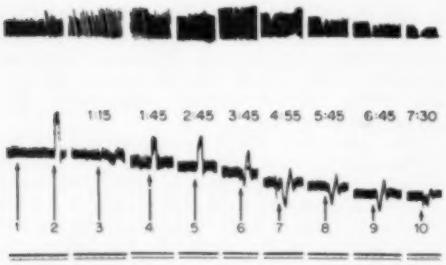

Fig. 1.—Hypotension in a normal dog after i. v. injection of 1.0 mg./Kg. of alseroxylon alkaloids: *1*, normal tracing—B. P. 160 mm. Hg; *2*, carotids clamped—B. P. 160 to 220 mm. Hg; *3*, 1.0 mg./Kg. alseroxylon alkaloids i. v.; *4*, *5*, *6*, *7*, *8*, *9*, *10*, carotid clampings; *4*, B. P. 142–176 mm. Hg; *5*, B. P. 136–176 mm. Hg; *6*, B. P. 126–156 mm. Hg; *7*, B. P. 110 to 80 to 130 mm. Hg; *8*, B. P. 105 to 82 to 118 mm. Hg; *9*, B. P. 94 to 72 to 112 mm. Hg; *10*, B. P. 92 to 80 to 95 mm. Hg. Upper tracing, respiration; lower tracing, time in minutes.

The typical, slowly developing but progressive reduction in blood pressure and heart rate was observed in all of the animals of the four groups. These effects usually became evident within one-half hour. In the majority of the animals of all the groups, the blood pressure and heart rate appeared to have become fairly stabilized by the sixth hour of observation. The respiration was not critically observed or analyzed, but definitely became slowed in rate, and usually reduced in depth in the majority of the animals.

In none of the normal dogs, in several of the vagotomized and carotid denervated animals, and in all of the completely denervated dogs, the injection of the alseroxylon alkaloidal preparation caused a slight rise in the mean arterial pressure which persisted for approximately twenty minutes. After one-half hour, the blood pressure returned to normal or below, whereupon it continued to fall in the typical Rauwolfia fashion.

When the alseroxylon-induced hypotension became more pronounced as the experiment progressed, the usual carotid occlusion response became diphasic as well as somewhat delayed (Figs. 1 and 2). The usual 45-second occluding period now produced only a depressor response. With continued occlusion beyond 45 seconds, a pressor response always ensued. At the last observation (at the end of six hours) when the mean arterial pressure had reached a relatively low nadir, both phases of this response were considerably lessened or almost completely blocked.

As a sequence to the above four groups of animals, several additional animals were experimented upon in an effort to shed more light on the problem at hand.

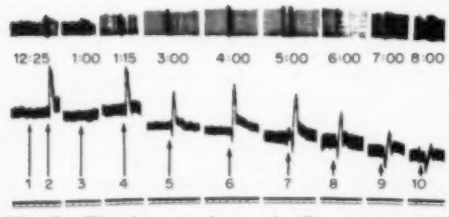

Fig. 2.—The hypotension and effects on carotid clampings produced in a vagotomized dog by intravenous injection of 1.0 mg./Kg. of the alseroxylon alkaloids: *1*, normal tracing—B. P. 150 mm. Hg; *2*, carotids clamped—B. P. 150 to 220 mm. Hg; *3*, alseroxylon—1.0 mg./Kg. i. v.; *4*, *5*, *6*, *7*, *8*, *9*, *10*, carotids clamped; *4*, B. P. 150 to 220 mm. Hg; *5*, B. P. 122 to 175 mm. Hg; *6*, B. P. 114 to 188 mm. Hg; *7*, B. P. 100 to 168 mm. Hg; *8*, B. P. 95 to 140 mm. Hg; *9*, B. P. 84 to 110 mm. Hg; *10*, B. P. 80 to 88 mm. Hg. Note diphasic response from 5 through 10. Upper tracing, respiration; lower tracing, time in minutes.

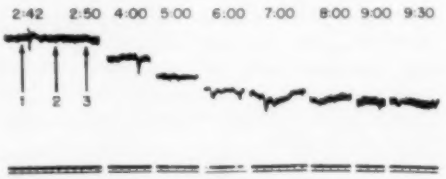

Fig. 3.—Hypotension following intravenous administration of 1.0 mg./Kg. of alseroxylon alkaloids in dog with complete carotid area denervation and intact vagi. *1*, *2*, carotid clampings—no change in blood pressure; *3*, 1.0 mg./Kg. of alseroxylon alkaloids, B. P. 194 mm. Hg; 4:00, B. P. 160 mm. Hg; 5:00, B. P. 134 mm. Hg; 6:00, B. P. 112 mm. Hg; 7:00, B. P. 106 mm. Hg; 8:00, B. P. 100 mm. Hg; 9:00, B. P. 96 mm. Hg; 9:30, B. P. 92 mm. Hg. Lower tracing, time in minutes.

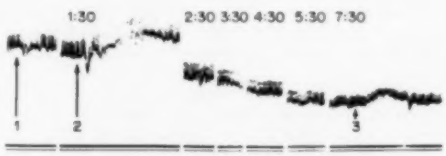

Fig. 4.—Hypotension following intravenous administration of 1.0 mg./Kg. of alseroxylon alkaloids in a dog with the vagi and carotid bifurcations completely denervated. *1*, carotids clamped—no blood pressure change; *2*, alseroxylon, 1.0 mg./Kg. i. v. at 1:30, B. P. 164 mm. Hg; between 1:30 and 2:30, B. P. 164 to 204 mm. Hg; 2:30, B. P. 120 mm. Hg; 3:30, B. P. 114 mm. Hg; 4:30, B. P. 98 mm. Hg; 5:30, B. P. 80 mm. Hg; 7:30, B. P. 74 mm. Hg; *3*, 2.6 cc. of 0.75% citric acid vehicle given i. v., B. P. 74 to 95 mm. Hg. Lower tracing, time in minutes.

Because all of the completely denervated dogs showed a transient increase in blood pressure immediately following the injection of the alseroxylon alkaloids, one completely denervated animal was given a series of injections of the appropriate volume of the citric acid vehicle alone. Following all of these injections, the blood pressure rose 10 to 20 mm. Hg and returned to its original level within 15–20 minutes. As shown in Fig. 2 (arrow No. 3), the vehicle alone also produced a slight elevation in the blood pressure when injected at the end of the observation period in an alseroxylon treated animal.

TABLE I.—THE REDUCTION IN HEART RATE IN FOUR GROUPS OF DOGS AFTER THE INTRAVENOUS ADMINISTRATION OF 1.0 MG./KG. OF THE ALSEROXYLON FRACTION OF RAUWOLFIA ALKALOIDS

Animal Group	No. of Animals	Heart Rate/min. Pre-Inj. Mean and S. D.	Heart Rate/min. Sixth-Hr. Mean	Heart Rate/min. Mean Absolute Diff. and S. D.
Normal	3	174 ± 14	145 ± 22	29 ± 3
Vagotomized	5	182 ± 28	146 ± 4	36 ± 24
Carotid denervated	5	184 ± 15	153 ± 10	31 ± 12
Completely denervated	5	170 ± 17	142 ± 9	28 ± 17

In this instance, the blood pressure rise was considerably less than that which occurred during the injection of the alseroxylon alkaloids.

One mg./Kg. of the alseroxylon alkaloids was injected intravenously into two vagotomized dogs in which the pressoreceptors were denervated leaving the carotid bodies intact. In both cases the qualitative and quantitative blood pressure effects were similar to those recorded in the completely denervated animals.

One vagotomized dog was given a total of 3.0 mg./Kg. of the alseroxylon alkaloids intravenously. After six hours the blood pressure had fallen to 94 mm. Hg which compares favorably with the values obtained in the vagotomized animals of Table II and indicates that 1.0 mg./Kg. of the alseroxylon produces maximum blood pressure effects.

In concluding the results, mention can be made of some additional information which was obtained in two of the normal animals but which was not included in Tables I, II, and III. In animal 1, the blood pressure fell three more millimeters, or to 107 mm. Hg by the end of seven hours. Both vagi were sectioned at this point. The blood pressure quickly rose to 120 mm. Hg and slowly receded to 104 mm. Hg after two more hours. The heart rate remained the same but the effects of carotid occlusion were slightly augmented after the vagi were sectioned. In normal animal 2, the vagi were sectioned after the sixth hour. After two additional hours, the blood pressure had not changed while the heart rate was slightly lower than before vagotomy.

DISCUSSION

It would doubtless have been more ideal to have included more animals in each of the groups studied, as well as to have included a like number of control animals for each of the surgically different groups of animals. However, the desirability to proceed to a study of the effect of the alseroxylon alkaloids when injected directly into the carotid receptor areas, as well as the cost of running additional animals in this experiment, made the employment of more treated and control animals prohibitive. Nevertheless, the results do depict a definite trend of action for the Rauwolfia alkaloids which, when analyzed and compared with information already existing in the literature, permit the deduction of several definite conclusions.

As can be seen from Table I, the degree of bradycardia was approximately the same regardless of the surgical backgrounds of the animals of the various groups. These values indicate that the vagi and carotid sinus nerves are responsible for little, if any, of the alseroxylon-elicited bradycardia. The data obtained in the two normal dogs which were vagotomized after the sixth and seventh hours, respectively, offer further support to this viewpoint. Two hours after the vagotomy in these two dogs, the heart rate was somewhat lower in one animal, and remained the same as before vagotomy in the other.

These observations leave the ultimate mechanism of the alseroxylon-induced bradycardia to a direct cardiac depressant action or to a decreased

TABLE II.—THE FALL IN BLOOD PRESSURE IN FOUR GROUPS OF DOGS AFTER THE INTRAVENOUS ADMINISTRATION OF 1.0 MG./KG. OF THE ALSEROXYLON FRACTION OF RAUWOLFIA ALKALOIDS

Animal Group	Dog	Mean Arterial Pressures (mm. Hg) Pre-Inj.	Pre-Inj. Mean	After 6 hr.	After 6 hr. Mean	Absol. Diff.	Absol. Diff. Mean	Diff., %	Mean Diff., %
Normal	1	162	150	110	102	−52	−56	32	36
	2	156		92		−64		41	
	3	158		105		−53		34	
Bilateral cervical vagotomy	4	151	144	108	93	−43	−50	28	35
	5	145		84		−61		42	
	6	146		94		−52		36	
	7	146		96		−50		34	
	8	130		84		−46		35	
Bilateral carotid sinus denervation	9	194	184	96	96	−98	−88	51	48
	10	185		105		−80		43	
	11	192		86		−106		55	
	12	174		100		−74		43	
	13	174		92		−82		47	
Carotid sinus denervation plus vagotomy	14	224	170	86	77	−138	−93	62	54
	15	144		72		−72		50	
	16	164		74		−90		55	
	17	154		74		−80		52	
	18	164		78		−86		52	

TABLE III.—THE BLOOD PRESSURE RESPONSES TO BILATERAL CAROTID ARTERY OCCLUSION FOLLOWING THE INTRAVENOUS INJECTION OF 1.0 MG./KG. OF ALSEROXYLON ALKALOIDS IN NORMAL AND VAGOTOMIZED DOGS

		Before Inj. of Drug	After Injection of Alseroxylon					
			1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.
Bilateral carotid occlusion responses, mm. Hg	Normal animal							
	1	168–230	134–160	120–138	126–150	120–170	116–140	110–142
	2	160–220	142–176	136–176	126–156	110–130	105–118	92–95
	3	158–210	128–160	120–160	126–158	116–154	110–140	105–134
	Vagotomized animal							
	4	148–222	141–218	126–188	...	118–174	116–158	108–158
	5	150–220	...	122–175	114–188	100–168	95–140	84–110
	6	140–212	140–230	120–195	102–150	97–132	96–142	94–138
	7	146–216	128–210	110–200	108–164	96–150	86–140	96–150
	8	132–204	...	...	...	...	80–96	84–100

central sympathetic tone. In this respect, reserpine, which is one of the major components of the alseroxylon alkaloids, has been shown to produce a decrease in activity in the isolated hearts of cats and rabbits, but only after comparatively long duration of action (10). In intact cats, oscillographic records showed a diminution of the sympathetic outflow in the sympathetic cardiac nerve occurring simultaneously with bradycardia. In this same animal, excitation of the vagal system was not likely since no component inhibitable by atropine could be demonstrated, and division of the vagus had no influence on the effect (3).

An analysis of the blood pressure values (Table II) reveals that a considerably greater absolute difference in the blood pressure readings before and six hours after the alseroxylon alkaloids were injected occurred in the last two animal groups (carotid denervated and completely denervated animals) than in the first two groups (normal and vagotomized animals). The mean percentage difference was accordingly also considerably larger in the last two animal groups. Little significance can be drawn from these values, however, because of the larger preinjection blood pressure readings which also prevailed in these latter two groups.

Of greater importance than the absolute or mean percentage differences are the actual blood pressure readings which were recorded at the end of the six-hour observation period. The values so obtained differed little in the first three animal groups, being on the average 102, 93, and 96 mm. Hg for the normal, vagotomized, and carotid denervated groups, respectively, as shown in Table II. The average value similarly obtained for the completely denervated animals, however, was only 77 mm. Hg.

Several factors which lend credence to the deductions that the lower blood pressure levels in the last group are real and not just due to chance are the following: (*a*) The blood pressure values of all the animals in this group with but one exception were below 80 mm. Hg after six hours. The exception was doubtless a hypertensive dog which had a preinjection level of 224 mm. Hg. (*b*) Not one of the 13 animals of the first three groups showed a final blood pressure reading that was below 84 mm. Hg, while most of these animals had pressures which were considerably higher than this value, as can be seen in Table II. (*c*) In the two normal animals which were vagotomized after the sixth and seventh hours, respectively, and which were observed for two additional hours, the final blood pressures were still only down to 104 and 92 mm. Hg, respectively. (*d*) The sixth hour blood pressure reading in the vagotomized dog which had been given a total of 3.0 mg./Kg. of the alseroxylon alkaloids was down to only 94 mm. Hg. (*e*) Both dogs which were completely denervated except for intact chemoreceptors displayed blood pressures below 80 mm. Hg just as the completely denervated animals had shown after six hours. (*f*) The blood pressure levels in all of the animals of all of the groups had reached a relatively stable nadir by the end of six hours, indicating that no major changes in the readings attributable to the drug could have been anticipated had the animals been observed for longer periods of time.

The major significance that can be attached to the above observations is that neither the vagi nor the carotid sinus nerves alone are of any import in the inducement of the hypotension elicited by the intravenous administration of the alseroxylon alkaloids in dogs. When both or either of these nervous pathways are intact, however, they definitely do play a part, at least in the acute experiment, as evidenced by the lower blood pressure levels exhibited when they are both denervated. This role which they play when intact is not one which contributes to the hypotension, however. Instead, it is in the nature of a buffering action which is effective in keeping the blood pressure from falling to the lower levels which ensue when both of these nervous pathways are surgically interrupted.

This conclusion is in line with the observed blood pressure levels of the four groups of animals studied and is also in harmony with the normal physiological functions purported for the carotid sinus and vagi nerves. Hence, in the normal animals, both buffer mechanisms were at work in an effort to prevent the alseroxylon hypotension. In the vagotomized animals, the carotid sinus nerve acted as the sole buffer, while in the carotid denervated groups, the vagi assumed the buffer role. But when both buffer mechanisms were denervated, the alseroxylon-elicited hypotension no longer was held under so strict abeyance. Consequently, the blood pressure in this latter group of animals continued to fall until it was between 70 and 80 mm. Hg, this final level

being solely determined by the hypotensive potency of the alseroxylon alkaloids acting by some mechanism other than through the vagi or afferent carotid area nerves.

That the carotid chemoreceptors play no role in the alseroxylon hypotension is indicated by the fact that the blood pressure fell to the same lower levels regardless of whether they were intact or denervated. This fact is verified in the second experiment which is reported elsewhere in THIS JOURNAL.

The transient hypertension seen immediately following the injection of the alseroxylon alkaloids in all of the completely denervated dogs and in a few of the vagotomized and carotid denervated dogs was doubtless due to the acidity and volume of the vehicle, the latter of which varied according to the size of the dog. This was shown by the fact that the appropriate volume of vehicle alone produced exactly the same type of blood pressure curve. This phenomenon was not seen in any of the normal animals because both buffer mechanisms had an over-riding effect; was seen in only certain of the partially denervated dogs because of the variation in potency of the remaining buffer nerves in the different dogs; and was always seen in the completely debuffered animals because of the complete absence of the normal compensatory nervous pathways.

The progressive reduction of the carotid occlusion reflex response following alseroxylon administration has been previously demonstrated and discussed by other investigators (1, 2, 6).

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Rauwolfia Hypotension II.*

Action of the Alseroxylon Alkaloids and Epinephrine on the Carotid Pressoreceptors

By CARL A. SCHLAGEL† and JOHN W. NELSON‡

The action of the alseroxylon alkaloids when injected into the adventitial layer of the carotid bifurcations of vagotomized dogs was investigated. The results showed, as a previous investigation had already indicated, that the Rauwolfia hypotension is primarily mediated through the central nervous system and secondarily through a direct muscular vasodilatory action of these alkaloids. This hypotension is limited in degree and almost devoid of any postural hypotensive aspects because of the incomplete nature of the central blocking action together with the vasodilatory action of the alseroxylon on the carotid sinuses whereby the normal physiological activity of these buffering pressoreceptors is potentiated. A pronounced pressor activity of carotid adventitially-injected epinephrine seen in orally treated Rauwolfia dogs is due to: (*a*) the preexisting hypotension and the Rauwolfia-induced blocking of the central components of the carotid reflex arc; (*b*) the diffusion or otherwise leaking of a portion of the epinephrine dosage into the general circulation; and (*c*) to the action of this systemic epinephrine on the effector end organs which have become sensitized by a Rauwolfia-engendered (pharmacologically-induced) sympathectomy.

IN A PREVIOUS ARTICLE in THIS JOURNAL, it was shown that the hypotension elicited by the alseroxylon alkaloids in dogs was not mediated through either the vagal or carotid nervous pathways. However, these observations did not entirely eliminate these nerves from further consideration as two of the possibly many sites of Rauwolfia action because it was also shown that when these nerves were intact, the alseroxylon hypotension was less intense or somewhat moderated in degree. Whether this buffering action was due solely to the normal function of the sinoaortic receptors reacting to the abnormal reduction in blood pressure, or also to a secondary local action of the alkaloids acting directly on these receptors was not studied in the previous experiment. Accordingly, the present investigation was designed to test the action of the alser-

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† Fellow of the American Foundation for Pharmaceutical Education. Present addres: The Upjohn Company, Kalamazoo, Mich.
‡ Present address: College of Pharmacy, The Ohio State University, Columbus 10.
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oxylon alkaloids when administered locally at the carotid bifurcation. Such a study should add weight to the previous investigation, and should give some clues as to why the Rauwolfia preparations are only mildly acting antihypertensive agents. It should also help explain the usual absence of postural hypotension following Rauwolfia therapy.

EXPERIMENTAL

The animals employed, the surgical techniques used, and the methods for recording blood pressure and carotid occlusion reflex responses have been described elsewhere in THIS JOURNAL. In preliminary trials utilizing 10 dogs, an aqueous extract of *Rauwolfia serpentina*, a commercial solution of epinephrine 1:1000 (Parke-Davis), and a 0.2% solution of $NaNO_2$ in normal saline were used. The Rauwolfia preparation had previously been shown in this laboratory to possess immediate, potent, and prolonged hypotensive activity when injected intravenously in dogs (1). It was used to attempt to verify the suspected Rauwolfia potentiating effect on the pressor-buffering action of the carotid receptor areas and in lieu of the alseroxylon alkaloids and reserpine in order to conserve the limited supply of these latter alkaloids. The epinephrine hydrochloride and $NaNO_2$ were chosen because of their known action on the carotid receptors when injected locally at the carotid bifurcation.

These preliminary trials were also primarily conducted in an effort to determine the final experimental design to be employed in the main body of this study. Accordingly, the blood pressure effects of the above three drugs were compared by applying the drugs to the carotid bifurcations with cotton swabs, by injecting 1.5-ml. volumes of the same drugs into each of the carotid conjunctival spaces, or by injecting 0.1 ml. of the preparations directly into the adventitial layers of the carotid arteries at the region of the bifurcations. In addition, the above three drugs were administered by the above three routes in normal dogs (all nerves intact), in vagotomized dogs, and in vagotomized and unilateral carotid denervated animals. In the latter case, the drugs were administered to only the remaining intact carotid area.

In all instances, the blood pressure changes with the Rauwolfia extract (pressor response) with epinephrine (depressor), and with $NaNO_2$ (pressor) were analogous qualitatively. The quantitative changes, however, were less erratic and usually more potent only following the intra-adventitial injection technique in the vagotomized animals. Unilateral carotid denervation with contralateral drug injection offered no particular advantage. Therefore, in the remainder of this experiment, all the animals were vagotomized in order to limit the reflex regulation of blood pressure to the carotid receptor areas. Likewise, all the drugs, unless otherwise specified, were injected in a total volume of 0.1 ml. into the adventitia of each of the carotid arteries at the region of the bifurcations. The injections were made with a 0.25-ml. syringe using a 27-gauge needle made considerably thinner by filing. The actual technique involved injecting one-half the volume (0.05 ml.) into the adventitia of both the lateral and medial aspects of each carotid receptor area giving a total of four injections for each single administration of drug.

Part I.—Various doses of the alseroxylon alkaloids were injected bilaterally into the carotid areas and their effects on the blood pressure noted. Because these results, as well as the above preliminary observations with the aqueous Rauwolfia extract, showed a pressor action on the carotid receptors which suggested a possible adrenolytic activity, 500-mcg. doses of the alseroxylon alkaloids were injected into the carotid areas both alone, before, and following similarly injected 100-mcg. doses of epinephrine always as the hydrochloride. In a majority of the dozen dogs of this section, 0.1-ml. doses of the citric acid vehicle were injected toward the termination of each experiment. An additional animal received several 0.1-ml. doses of the vehicle alone. Reserpine was studied alone in two animals and together with the alseroxylon alkaloids in another animal for comparison purposes.

Part II.—To study further the pressor response that always occurred following the carotid adventitial injection of appropriate doses of the alseroxylon alkaloids as shown in the results of Part I, four dogs were given 0.5 mg./Kg. of the alseroxylon fraction orally each day for a period of ten days. Two other dogs received 50.0 mcg./Kg./day of reserpine in an identical manner. The tenth dose was given two hours before the administration of the anesthetic on the day the animal was experimented upon. All these animals were then vagotomized and the effects of carotid occlusion and injection of various drugs such as the alseroxylon alkaloids, epinephrine, $NaNO_2$ and vasopressin (Parke-Davis) into the carotid bifurcation were noted. In one of the above orally-treated reserpine animals, a comparison was made between the blood pressure effects of epinephrine injected intravenously and into the carotid area both before and after an appropriate epinephrine reversing dose of Dibenamine®. The effects of carotid area injections of epinephrine and the alseroxylon alkaloids were also studied in one dog which had been given 1.0 mg./Kg. of the alseroxylon fraction six hours previously, and in which the carotid occlusion pressor reflex was blocked to a similar degree as that observed in the orally treated animals.

RESULTS

Part I.—The bilateral injection of doses of 1.0 mcg. of the alseroxylon alkaloids produced no changes in the blood pressure whatsoever. Doses of 10, 25, 50, and 100 mcg. produced only slight pressor responses (5 to 20 mm. Hg), which were transient in nature.

Because of a difference in solubility, only 50 mcg. of reserpine could be dissolved in 0.1 ml. of the citric acid vehicle. When this dose was injected into each sinus region, either no change in blood pressure occurred, or, in a few instances, a slight but transient hypertension was recorded. In another dog, 150 mcg. of reserpine dissolved in 0.3 ml. of vehicle was injected into each sinus region. A 10 mm. Hg rise in pressure occurred after the first dose, while no change was observed after the second, similar dose. The same doses of reserpine and the alseroxylon alkaloids (150 mcg. in 0.3 ml.) were then given at

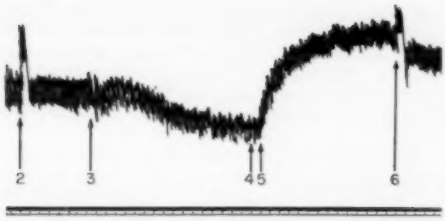

Fig. 1.—Effects of carotid adventitially-injected epinephrine and alseroxylon alkaloids on the blood pressure of a vagotomized dog. Male dog, 16.6 Kg.; lower tracing: time in minutes. *2*, 4:00 carotids clamped, B. P. 148 to 206 mm. Hg; *3*, 4:23, epinephrine, 100 γ into each carotid area, B. P. 144 to 96 mm. Hg; *4*, carotids clamped at 4:39, no change in B. P.; *5*, 4:40, 500 γ of alseroxylon in each carotid area, B. P. 96 to 208 mm. Hg; *6*, effects of carotid clamping restored, B. P. 208 to 228 mm. Hg.

different intervals in still another animal. The reserpine raised the blood pressure only 6.0 mm. Hg, while the alseroxylon alkaloids produced a 20.0 mm. Hg elevation.

Following the bilateral injection of 250 to 500 mcg. of the alseroxylon alkaloids, almost immediate and rather large elevations in the blood pressure occurred. The pressure remained elevated for some time, usually at least one hour, and in several instances, for two hours or longer. During the hypertensive period, the carotid occlusion reflex response was usually not blocked completely but was considerably depressed percentage-wise due to the high blood pressure level previously established by the Rauwolfia alkaloids.

All of the results obtained with the 500-mcg. doses of alseroxylon and 100-mcg. doses of epinephrine were the same qualitatively but varied quantitatively depending upon the exact sequence of dosage employed. For instance, when epinephrine was injected initially, the blood pressure always fell to a level at which the carotid occlusion reflex was blocked. When given during the pressor response to the alseroxylon alkaloids, the ensuing hypotension was very much less impressive, belated, or did not occur at all. On the contrary, when the alseroxylon alkaloids were administered during the nadir of an epinephrine response, the effects of epinephrine were reversed as shown in Fig. 1 (blood pressure elevated to a level higher than the pre-injection norm), or the blood pressure was simply returned to its pre-epinephrine level. In every instance, the alseroxylon alkaloids were able to re-establish the carotid occlusion response which had previously been blocked by action of the epinephrine.

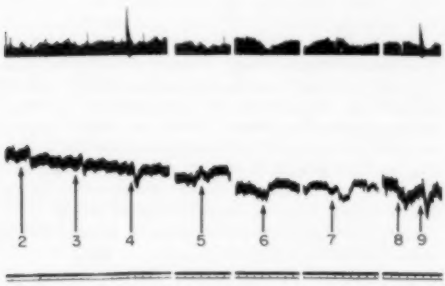

Fig. 2.—Lack of blood pressure response following carotid adventitially-injected epinephrine and alseroxylon alkaloids on a vagotomized dog with the carotid pressoreceptors selectively denervated, leaving the carotid bodies intact. Female dog, 9.3 Kg.; upper tracing: respiration; lower tracing: time in minutes. *2* and *3*, carotids clamped, little change in B. P. indicating pressoreceptors denervated; *4*, NaCN, 1.0 mg./dog i. v., hyperpnea indicating chemoreceptors intact; *5*, alseroxylon, 500 γ in each carotid area, little change in blood pressure; *6*, epinephrine, 100 γ in each carotid area $1^1/_2$ hr. after *5*, little change in blood pressure; *7*, alseroxylon repeated 20 min. after *6*, little change in pressure; *8*, carotids clamped 1 hr. after *7*, little change in pressure; *9*, NaCN, 1.0 mg./dog i. v., hyperpnea indicating chemoreceptors still active and intact.

The carotid adventitial injection of 500 mcg. of alseroxylon in the two dogs which were completely denervated with the exception of having intact chemoreceptors produced no change in the blood pressure as shown in Fig. 2. A total of four such doses was administered to these two animals.

The injection of several doses of the vehicle alone (0.75% citric acid) in the majority of the above animals was without effect. The vehicle was also without effect when given to one animal which had received no previous medication other than the anesthetic. That the carotid sinuses were responsive in this animal was shown by the later injection of the alseroxylon alkaloids and by occlusion of the carotid arteries.

Part II.—The blood pressures and heart rates of the six orally treated and vagotomized animals are shown in Table I. These values were obtained before any drugs were injected into the carotid sinus areas.

TABLE I.—THE MEAN ARTERIAL BLOOD PRESSURES AND HEART RATES OF SIX VAGOTOMIZED DOGS WHICH HAD RECEIVED DAILY ORAL DOSES OF THE RAUWOLFIA ALKALOIDS FOR A PERIOD OF TEN DAYS

Animal	Drug	Dose/day	M. A. P. (mm. Hg)	H. R. (beats/min.)
1	Alseroxylon alkaloids	0.5 mg./Kg.	120	120
4	Alseroxylon alkaloids	0.5 mg./Kg.	90	110
4	Alseroxylon alkaloids	0.5 mg./Kg.	96	104
4	Reserpine	50 mcg./Kg.	93	118
5	Alseroxylon alkaloids	0.5 mg./Kg.	132	98
6	Reserpine	50 mcg./Kg.	131	110

Other results obtained in the four alseroxylon treated and in one of the reserpine animals were as follows: (*a*) an almost complete blocking of the hypertensive response which is reflexly elicited in normal animals by occlusion of the carotid arteries; (*b*) an almost complete blocking of the hypertensive response which is reflexly elicited in normal animals by the carotid adventitial injection of 500 mcg. of the alseroxylon alkaloids; (*c*) a very marked rise in the arterial pressure following

the injection of 100 mcg. of epinephrine into the adventitia of the carotid bifurcations (this is opposed to the depressor response elicited by similarly injected epinephrine in normal animals); similar but not identical responses when 100 mcg. of epinephrine was injected into the carotid bifurcation as when 2–3 mcg. of epinephrine was administered intravenously; (*d*) almost identical elevations in the blood pressure when epinephrine was injected into the carotid area either before or after complete carotid area denervation. A record of one of these animals which depicts most of these responses is shown in Fig. 3.

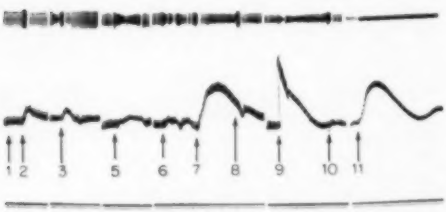

Fig. 3.—Blood pressure responses produced by carotid adventitially-injected alseroxylon and epinephrine in a vagotomized dog following a ten-day oral alseroxylon treatment at a dose of 0.5 mg./Kg./-day. Female dog, 11.0 Kg.; upper tracing: respiration; lower tracing: time in minutes. *1*, normal tracing, B. P. 92 mm. Hg; *2*, *3*, carotids clamped twenty-five minutes apart, carotid reflex response not quite completely blocked; *5*, *6*, alseroxylon, 500 γ into each carotid area twenty-five minutes apart, pressor responses similar to those elicited by carotid clamping; *7*, repeat *4* which is not shown, epinephrine, 100 γ into each carotid bifurcation, marked pressor response rather than depressor as in normal dogs; *8*, carotids clamped during fall in B. P., slight alteration in B. P.; *9*, epinephrine, 50.0 γ/dog i. v., very steep and more potent rise in B. P.; *10*, carotids clamped; *11*, epinephrine repeated as in *7*.

There are some other interesting aspects to this peculiar epinephrine-produced pressor response which were not mentioned in the above account. For instance, the same qualitative blood pressure response, but one of considerably less magnitude, could be obtained by application of epinephrine (1:1000) to the carotid sinus region with a swab. If the carotid sinus areas were denervated with phenol, or injected with 500 mcg. of the alseroxylon alkaloids, or if the carotids were occluded at the onset or during the fall in blood pressure which followed the pressor aspect of the epinephrine response, the blood pressure kept right on falling just as it would have had these experimental techniques not been instituted.

Pressor responses were also recorded following carotid area injections of 0.1 ml. (2 units) of vasopressin or 0.1 ml. of a 1% solution of ephedrine sulfate. However, these responses were of considerably less magnitude than those following epinephrine being on the average 24 mm. Hg for vasopressin and 22 mm. Hg for ephedrine sulfate. The pressor responses to these latter two drugs were also less sharp (delayed), but were progressive and persistent, sometimes for one hour or more. The intra-adventitial injection of 0.1 ml. of 0.2% $NaNO_2$, on the other hand, caused a fall in pressure of approximately 30 mm. Hg. This depressor response was also delayed (5–10 minutes) but persisted for an hour or more. In all instances, the degree of the pressor responses with vasopressin and ephedrine sulfate, and the depressor responses following $NaNO_2$ appeared to be dependent upon the pre-injection blood pressure level.

For comparing the responses in the long term, orally treated to the intravenously treated animals, 3.0 mg./Kg. of the alseroxylon alkaloids were administered intravenously to a vagotomized dog. After six hours, the blood pressure had only receded to 94 mm. Hg and the carotid occlusion was still capable of elevating the pressure 40 mm. or to 134 mm. Hg. The injection of 100 mcg. of epinephrine into the carotid area of this animal raised the blood pressure only from 94 to 100 mm. Hg after five minutes, whereupon it fell to 92 mm. Hg where it remained stationary for at least one-half hour.

Since it was apparent from this animal and from those reported upon in the previous article that 1.0 mg./Kg. given intravenously was capable of producing maximum blood pressure effect, 1.0 mg./Kg. was then given to another vagotomized animal by intravenous injection. After six hours the blood pressure was down to 84 mm. Hg and carotid occlusion was only effective enough to produce a 16-mm. rise.

The intra-adventitial injection of 100 mcg. of epinephrine at this point elevated the blood pressure only 10 mm. Hg. The pressure then receded to 70 mm. Hg which was approximately 12 mm. below the pre-injection level. After one hour, 400 mcg. of the alseroxylon alkaloids elevated the pressure from 70 to 82 mm. Hg or to the pre-epinephrine injection level. At this point, 150 mcg. of epinephrine elevated the pressure to 126 mm. Hg. One-half hour later the pressure was stabilized at 86 mm. Hg. Vasopressin and $NaNO_2$ in this animal produced an elevation and reduction in pressure, respectively, which was analogous to their effects in the orally pretreated animals.

The last animal studied in this experiment was a reserpine pretreated dog. Just as in the other orally treated animals, both intravenous and carotid adventitial injections of epinephrine produced similar pressor responses. However, when identical doses of epinephrine were again administered following an epinephrine reversing dose of Dibenamine, depressor responses were obtained which were almost mirror images of the pre-Dibenamine pressor blood pressure curves. The blood pressure tracing for this animal is shown in Fig. 4.

DISCUSSION

The pressor response which was always obtained following the injection of from 250 to 500 mcg. of the alseroxylon alkaloids into the adventitia of the carotid bifurcations shows that this Rauwolfia fraction has a very definite local action on the nervous receptors of this area. This pressor action is in complete agreement with the results of the previous study reported elsewhere in THIS JOURNAL, where it was shown that the carotid receptors displayed a definite pressor, buffering capacity following the intravenously administered alseroxylon alkaloids. Both of these studies likewise limit the site of this

Fig. 4.—Blood pressure responses following intravenous and carotid adventitially-injected epinephrine both before and after the i. v. injection of Dibenamine in a vagotomized dog which had previously been given 50 mcg./Kg./day of reserpine for a period of ten days. Male dog, 16.3 Kg.; upper tracing: respiration; lower tracing: time in minutes. *1*, Normal tracing, B. P. 131 mm. Hg; *2*, carotids clamped, B. P. 131 to 154 mm. Hg indicating carotid reflex almost completely blocked; *3*, epinephrine, 100 γ into each carotid area, B. P. 130 to 112 to 196 mm. Hg; *4*, epinephrine, 2.0 γ/Kg., i. v., similar but steeper rise in B. P. from 110 to 205 mm. Hg; (between *4* and *5*, 25 mg./Kg. Dibenamine given i. v. over a one-hour and ten-minute period); *5*, carotids clamped $1^1/_2$ hr. post Dibenamine, B. P. 140 to 154, smaller rise than 2 but to same summit; *6*, epinephrine, 2.0 γ/Kg. i. v. as in *4*, B. P. 140 to 50 mm. Hg showing epinephrine reversal; *7*, epinephrine 100 γ in each carotid area as in *3*, B. P. 140 to 50 mm. Hg showing leakage into blood stream and reversal; *8*, vasopressin, 1.0 cc. or 2 units into each carotid area, B. P. 96 to 140 indicating leakage into blood stream with no reversal effect.

pressor action to the carotid pressoreceptors because the carotid chemoreceptors as the sole remaining compensatory nerves could neither buffer the hypotension elicited by the intravenous alseroxylon fraction nor produce hypertension after the local injection of these alkaloids. The results with reserpine in this respect are inconclusive because this alkaloid was not studied intravenously, and its relatively insoluble nature prevented a study of its local action on the carotid bifurcation.

There are three possible mechanisms by which the locally administered alseroxylon alkaloids could produce their pressor response through the carotid pressoreceptors. These are: by a local anesthetic action on the receptor nerve endings or fibers therefrom; by an adrenolytic action; or by a direct vasodilatory effect.

Using the method of Bulbring and Wajda (2) on guinea pigs, the authors investigated the possible anesthetic action of alseroxylon. However, the results were inconclusive because of an indurating effect of the acid vehicle. An anesthetic mechanism can nevertheless be ruled out because there are reports in the literature that both the alseroxylon alkaloids (3) and reserpine (4) are devoid of such an activity.

Although the alseroxylon alkaloids produced a pressor response which in some respects resembled an adrenolytic type activity, such an action can also be ruled out when the blood pressure curves following the alseroxylon fraction or epinephrine when given alone or in various sequences are carefully scrutinized. For instance, the blood pressure elevations following initial doses of alseroxylon were always of considerable magnitude while the known adrenolytic agents, Hydergine®, ergotamine, Dibenamine®, and Regitine®, (5–8) usually produce only a very slight or almost imperceptible hypertension when injected alone. Furthermore, when epinephrine was injected during the hypertension induced by a previous alseroxylon injection, either no change in the blood pressure level was observed or it slowly began to recede. In contrast, epinephrine always causes additional hypertension (a reversal of its usual action) when injected following a previous dose of an adrenolytic agent (6, 7) which itself produces a slight blood pressure elevation.

By the process of elimination, the local action of the alseroxylon alkaloids on the carotid pressoreceptors is left to a direct vasodilatory mechanism. Such a direct action on the musculature of blood vessels is not an entirely new discovery for it has frequently been postulated by other workers. However, the results of all the investigators who have studied this mode of action are still conflicting, which is most likely due to the different species of animals employed, the different Rauwolfia preparations used, and to the precise experimental techniques employed. At any rate, it is our belief that such a direct vasodilatory activity does exist, and although it is of secondary importance to the main central site of action, it is nevertheless of a great deal of importance. Thus, it has been shown that even when relatively large doses of the alseroxylon alkaloids are given intravenously or orally over a period of time, the central blocking action of these alkaloids is never quite complete. This enables the direct vasodilatory action of the alkaloids to exert a dual effect by one and the same mechanism. First, this nonneurogenic vasodilation probably contributes to the degree of Rauwolfia hypotension, the major portion of which is of the centrally-induced neurogenic variety. Secondly, the normal physiological buffering capacity of the carotid afferent nerves is potentiated by the Rauwolfia engendered muscular relaxation which occurs in the arterial walls where the receptors for these nerves are located. This physiological, as well as pharmacologically-potentiated action is of course possible only because the central pathway of this reflex arc is never completely blocked. Its importance lies in the fact that it offers an explanation as to why postural hypotension is seldom seen following Rauwolfia therapy. Accordingly, this doubly-induced buffering action limits the degree of the Rauwolfia hypotension, and in so doing it prevents any extensive venous pooling of blood. Further, when the patient assumes an erect position and the blood pressure commences to fall below the Rauwolfia-induced level, the carotid receptors further reduce their "firing off" and the blood pressure is thereby elevated or more nearly maintained at the level originally produced by the relative degree of the central sympathetic block. Thus extensive postural hypotension will not occur.

As in the preceding discussion, the results obtained in the orally treated Rauwolfia dogs again implicate the often postulated but as yet nondelimitative central nervous system as the chief locus of action for the Rauwolfia alkaloids. While the present observations yield no new clues relative to pinpointing the exact anatomical site of this central blocking action, they do definitely reaffirm that it is due to an interruption of the efferent sympathetic nervous impulses that Rauwolfia produces the major portion of its hypotensive effects. This is not only

evident from the fact that in these orally-treated animals the carotid occlusion reflex response and the usual pressor response following carotid adventitially injected alseroxylon are blocked to exactly the same degree, but is especially evident from the powerful, pressor response to carotid adventitially-injected epinephrine in these same animals.

The peculiar epinephrine pressor response was at first thought to be adrenolytic in nature. However, the pressor curve was too sharp and too transient in character for a typical adrenolytic response. Furthermore, as will be shown later, a portion of all drugs when injected into the carotid adventitia gets into the blood stream (not by accidental injection) where it may exert its typical systemic effects. With this being true also for epinephrine, then the response should have been depressor rather than pressor if the oral Rauwolfia did exert a peripheral adrenolytic action. That the epinephrine pressor response was not due to accidentally inserting the needle into or otherwise rupturing many of the minute blood vessels of the area is evident from the fact that this pressor response was never seen in any of the normal animals, but only in the orally-treated Rauwolfia dogs.

With all seemingly logical mechanisms being exhausted, it was then assumed that some of the epinephrine dosage was possibly diffusing or otherwise slowly entering the systemic circulation and thereby exerting its usual pressor action. This was eventually shown to be the case, but of even greater import is the fact that it was due only to the action of the oral Rauwolfia alkaloids that the pressor response was observed in the first place. A brief recapitulation of some of the results at this time will perhaps be of some value in clarifying the situation.

In normal animals, the injection of epinephrine into the carotid adventitia produces hypotension by constricting the arterial walls where the pressoreceptors are located. This constriction simulates hypertension in the carotid sinus; the carotid afferents increase their impulse traffic; central sympathetic substrates are inhibited; sympathetic efferent outflow is reduced; and hypotension ensues. Following similarly injected epinephrine in the oral Rauwolfia dogs, the same events transpire, but no hypotensive response is seen because of the preexisting hypotension and central sympathetic blocking action of the previously administered Rauwolfia. Instead, a potent hypertensive response is obscerved because some of the epinephrine leaks into the blood stream where it exerts its effects on the effector end organs which have become sensitized to epinephrine by virtue of a Rauwolfia-engendered, pharmacologically-induced sympathectomy. This sensitization is analogous to that seen following surgical sympathectomy. It develops over the ten-day, oral Rauwolfia feeding period and is due to the persistent reduction of sympathetic efferent impulses which in turn is due to the central sympathetic block.

That both the sensitization of the sympathetic, effector end organs as well as leakage into the blood stream are necessary for the epinephrine pressor response can be seen from the following examples: (*a*) Six hours following the intravenous administration of the alseroxylon alkaloids, the degree of hypotension and carotid reflex blocking is similar to that seen in the orally-treated dogs. However, when epinephrine is injected into the carotid area of the former animals, only a very feeble pressor or depressor response ensues. A potent depressor response is not obtained because of the preexisting hypotension and/or the central block. A potent pressor effect is also lacking because the effector end organs are not yet sensitized to the epinephrine that does reach the blood stream. (*b*) In the orally-treated dogs, carotid adventitially-injected vasopressin and ephedrine sulfate each produced a mild but persistent hypertension due to leakage into the blood stream. This pressor effect was allowed to dominate because the hypotension which these drugs always elicit in normal animals was inhibited by the Rauwolfia block on the central components of the carotid reflex arc. On the other hand, this same blocking action prevented the usual pressor response to similarly injected $NaNO_2$ so that when this drug diffused into the blood stream, only hypotension was seen.

In the last orally-treated animal, the carotid injection of epinephrine produced hypertension before Dibenamine and a mirror image hypotension after an epinephrine-reversing dose of Dibenamine. Ephedrine sulfate which cannot be reversed by Dibenamine still produced only hypertension in this animal. These results prove conclusively that Rauwolfia, unlike Dibenamine, possesses no peripheral adrenolytic action, and that portions of the dose of all drugs when injected into the adventitial layer of the carotid artery enter the blood stream to exert their usual systemic effects. If Rauwolfia possessed an adrenolytic action, the direction of the blood pressure changes following the intravenous epinephrine would have been the same both before and after Dibenamine. Likewise, if the epinephrine had not gotten into the blood stream when injected into the carotid area, the reversal would not have been observed following the Dibenamine.

The major significance of the observation that a portion of all drugs when injected or otherwise applied at the carotid area easily gain access to the blood stream is that the vasomotor changes induced in such studies must be more carefully analyzed, because the changes so induced are not brought about by a purely carotid reflex mechanism, but, instead, by the algebraic sum of the vasomotor changes mediated through the carotid reflex arc and by systemic action. Since the blood pressure changes from these two routes of administration are almost always in the opposite direction, the true potency of drugs acting locally on the carotid pressoreceptors can probably never be precisely determined. At best, only relative values may be obtained.

In conclusion, a few remarks with regard to the inactive nature of the citric acid vehicle when injected into the carotid area are in order. Previous workers have shown that a low *p*H may not affect the carotid pressoreceptors (9) as shown by action potential studies with epinephrine HCl solutions which have a *p*H of approximately 3–4 and with an HCl solution of *p*H 2.7. The inert or only feeble action of the vehicle on the carotid chemoreceptors is more difficult to explain. It is perhaps due to the fact that these receptors may already be at least partially stimulated by the respiratory depressing effects of the anesthetic and the Rauwolfia. The citric acid also may be unable to reach the chemoreceptors if these are intracellularly located which would be analogous to the permeability factors which are responsible

for the failure of certain microorganisms to take up citric acid in bacterial physiology studies on metabolism.

SUMMARY AND CONCLUSIONS

1. Results from the injection of 250 to 500 mcg. of the alseroxylon alkaloids into the adventitia of the carotid arteries at the region of the bifurcations show that these alkaloids are capable of pharmacologically potentiating the pressor buffering action of the carotid pressoreceptors against a simultaneous, centrally induced, Rauwolfia hypotension. The citric acid vehicle alone or the carotid chemoreceptors do not participate in this action.

2. This action is thought to be due to a direct vasodilatory action of the alseroxylon alkaloids upon the carotid musculature rather than to an anesthetic or adrenolytic effect. It produces vasomotor changes which are opposite in direction to similarly injected, small doses of the veratrum alkaloids. Accordingly, clinical combinations of the veratrum alkaloids and alseroxylon, while doubtless more potent hypotensively, would, by nature of the veratrum component, eliminate the potentiating, pressor, buffering action of the alseroxylon alkaloids which is important when orthostatic hypotension is considered.

3. The usual hypertension and hypotension following the respective intravenous administration of the commonly used pressor (epinephrine, vasopressin, ephedrine sulfate) and depressor agents ($NaNO_2$) are reversed to hypotension and hypertension when these drugs are injected into the adventitia of the carotid pressoreceptors of normal dogs. Further, when these same agents are injected into the carotid adventitia of oral, Rauwolfia pretreated animals, the vasomotor changes are reconverted so that the direction of the blood pressure changes are now identical to those observed following their intravenous administration. This reconverted vasomotor response is due to the blocking action of Rauwolfia on the central substrates of the carotid reflex arc together with a portion of the injected dose gaining access to the blood stream where it exerts a typical systemic effect.

4. Evidence is presented which reaffirms and extends the concept that the major hypotensive mechanism of the Rauwolfia alkaloids is through a reduction in efferent sympathetic outflow.

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Prolonged Effect of Riboflavin: Aluminum Monostearate Suspensions in Man*

By A. BRZEZINSKI, Y. M. BROMBERG, and F. G. SULMAN

The use of riboflavin suspensions with 2% aluminum monostearate for depot injection therapy has been studied in three patients. A depot of 150 mg. riboflavin takes up to 6 weeks to be absorbed, and smaller quantities take correspondingly shorter periods (100 mg., 5 weeks; 50 mg., 4 weeks). Control patients who received similar doses of riboflavin solutions showed return to normal riboflavin excretion in the urine within 3–4 days. The method of riboflavin administration in suspensions is much easier than the implantation of pellets previously described by us. The depots formed produce no local reactions and allow slow and continuous absorption of the vitamin from the site of injection.

NUTRITIONAL STUDIES in Israel have shown that riboflavin deficiency is very frequent (1) and that its incidence is much greater than that of other vitamin deficiencies (2). Clinical manifestations of this deficiency, such as glossitis, cheilitis, and corneal vascularization, are particularly common during pregnancy. Thus reduced excretion of riboflavin (averaging 95 mcg./liter of urine compared with 360 mcg./liter in normal pregnant women) was found in 21 per cent

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of 900 pregnant women (3). Riboflavin deficiency is also associated with prematurity of the fetus, a high incidence of antenatal death and postpartum agalactia or hypogalactia (4). Surprisingly enough, these clinical manifestations appeared not only in poorer classes in which riboflavin intake was inadequate, but also in well-nourished subjects suffering from chronic intestinal disturbances. The latter finding is principally due to inadequate absorption of this vitamin. The frequent occurrence of intestinal and hepatic disorders such as amebic dysentery, gastric achlorhydria, and hypochlorhydria, and various types of chronic hepatitis is undoubtedly the most important etiologic factor in production of deficiency symptoms (5).

Riboflavin therapy generally produces striking improvement in cases in which the deficiency is due to insufficient intake. On the other hand, poor results usually follow oral administration of riboflavin in subjects suffering from deficiency due to inadequate absorption. In these patients even parenterally administered riboflavin is eliminated from the body within a few days (6).

To maintain a constant level of riboflavin in tissues, we attempted implantation of riboflavin pellets so as to afford continuous and adequate absorption of the vitamin. This method seems particularly advantageous in patients who cannot be trusted to take medication regularly. We prepared pellets containing 50 mg. riboflavin fused with 50 mg. cholesterol (7) which on implantation maintained a high riboflavin level in man and animals for 45 days.

Recently we simplified treatment by developing a single injection technique. Two aspects of this type of therapy, the rate of excretion of riboflavin and the rate of recovery from the site of injection of a suspension of riboflavin aluminum monostearate in rats, have been reported (8).

In the present paper a report is given on treatment of three patients with suspensions of riboflavin aluminum monostearate in comparison with solutions.

METHODS

Riboflavin suspensions were prepared with 2% aluminum monostearate on which the riboflavin was previously absorbed.[1] They are stable when protected from light, easily tolerated, contain 50 mg. riboflavin per ml., and cause no local reaction on intramuscular injection.

Three patients, one suffering from riboflavin deficiency, the other two normal, received one i. m. injection of riboflavin suspension. The normal persons were injected first to observe their reaction: one received 50 mg. and the other 100 mg. riboflavin suspension. The riboflavin-deficient patient received 150 mg. riboflavin suspension. Three control patients received the same three doses of riboflavin in aqueous solution with 5% w/v sodium benzoate added as a solubilizer. All patients received a well-balanced diet in the hospital, with constant and normal content of riboflavin.

After a one week period of adaptation, the urine was assayed daily for two weeks for riboflavin. The patients were then given a single injection of riboflavin in suspension or solution and the excretion of the vitamin in the urine was determined daily by the fluorometric method (9). The urine was assayed at dilutions containing 0.1–0.2 mcg./ml. Excretion was expressed in terms of mcg. riboflavin excreted per day.

RESULTS

The average daily urinary excretion of the three patients injected once with riboflavin suspensions is shown in Fig. 1. Fig. 1 also shows that the patient who received 150 mg. riboflavin started from a subnormal level of riboflavin excretion. All patients had a prolonged curve of riboflavin excretion (Fig. 1). All patients tolerated the injection very

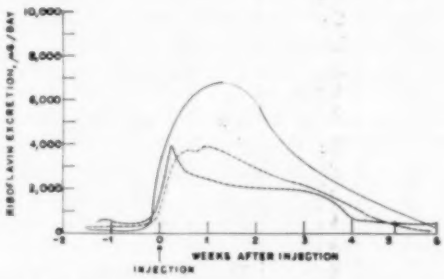

Fig. 1.—The excretion of riboflavin in mcg./day after injection of: 150 mg. in suspension — (return to normal level after 6 weeks); 100 mg. in suspension - - - (return to normal level after 5 weeks); 50 mg. in suspension ------ (return to normal level after 4 weeks).

well and did not show any untoward local reaction. The patient who suffered from riboflavin deficiency and who received 150 mg. of riboflavin suspension had a distinct clinical improvement of his deficiency symptoms during eight weeks of observation. The controls who received the riboflavin solutions returned within three to four days to a normal level of riboflavin excretion.

DISCUSSION

At first glance it seems that the method of injection of riboflavin suspensions described is not superior to the implantation method described by us earlier (7). Whereas 50 mg. riboflavin implanted provided riboflavin for 45 days, the same quantity injected as an aluminum monostearate suspension lasted up to 28 days only. This drawback can, how-

[1] We are indebted to Dr. G. Friedlaender of "Teva" Middle East Pharmaceutical & Chemical Works Ltd., Jerusalem (Israel), for the preparation of the suspensions and solutions.

ever, be easily made up by injection of 150 mg. of riboflavin suspension, the excretion of which lasts also 45 days. Higher doses are easily tolerated.

The results obtained with the suspension method are quite satisfactory. The present experiments were only carried out on three patients, the urine excretion of which was, however, studied daily to obtain clear results. Moreover, these results were corroborated by experiments in 24 rats in which the rate of absorption and excretion was studied thoroughly (8). The very slow absorption of the vitamin is not only due to slow liberation from the suspension, but also to poor solubility of riboflavin in body fluids, its solubility in an aqueous medium being 1:10,000.

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A Phytochemical and Pharmacological Study of *Onosma bracteatum* Wall.*

By P. C. DANDIYA and R. B. ARORA

A preliminary phytochemical and pharmacological investigation of *Onosma bracteatum* Wall. is presented. The actions of the dealcoholized detannated extract of the drug on cardiovascular system and smooth muscle have been studied. The extract lowers the blood pressure, depresses the heart, and possesses a spasmolytic action.

ONOSMA BRACTEATUM WALL. commonly known as "gaozavan" is a small herb growing in Kashmir and Kamaun at an elevation of about 11,500 ft. The leaves, flowers, and seeds are widely used in the indigenous system of medicine as acrid, cooling, and antipyretic and given in diseases of the chest and lungs, asthma, throat troubles, and stomatitis (1). Many practitioners of the Indian system of medicine are using the drug in cases of tachycardia, arrhythmias, hypertension, and auricular fibrillation.

In a preliminary report Arora and Dandiya (2) have shown that an extract of the drug is a myocardial depressant and produces fall in blood pressure.

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The authors are sincerely grateful to Dr. B. S. Kahali, Department of Physiology, S. M. S. Medical College, Jaipur, and to Dr. V. N. Sharma of this department, for their assistance in this study.

PREPARATION OF THE EXTRACTIVES

A mixture of leaves, fruits, and small stems (such as used by local ayurvedic practitioners) was pulverized to 20-mesh size, stray iron particles were removed by a magnet, and the whole thoroughly mixed.

Extractives.—Suitable, accurately weighed samples of the powdered drug were completely extracted by the methods of U. S. P. XIV (3), employing a Soxhlet continuous extraction apparatus with duplicate samples and ethanol 90%, ethanol 70%, distilled water, absolute ether, and petroleum ether were used as solvents. The extractives weighed as follows:

Ethyl alcohol (90%) extract	10.4%
Ethyl alcohol (75%) extract	13.2%
Water-soluble extract	21.3%
Ether-soluble extract	1.2%
Petroleum ether (60–80°) extract	3.2%

Alkaloids.—The immiscible solvent method of the U. S. P. XIV (3) was used as a basis for the qualitative test for alkaloids. Mayer's reagent, saturated solution of picric acid, 0.1 *N* iodine, and 10% tannic acid solution all failed to give a positive test or reaction.

Glycosides.—For extracting the glycoside a modification of the process by Rosenthaler (4) was employed. Five grams of calcium carbonate was added to one liter of 95% alcohol and 200 Gm. of the coarsely powdered drug was slowly added to the alcohol mixture kept boiling on a water bath. After all the drug had been added the boiling was continued for three hours under a reflux condenser. The mixture was filtered while hot and the drug refluxed again with hot alcohol. The combined hot alcoholic extracts were allowed to cool overnight. A small amount of yellowish white mucilaginous substance (*A*) settled down and was separated. The filtrate was concentrated to about 25 cc. at 20° and 20 mm. pressure. Some more yellowish substance (*B*) settled down. No crystallization, however, took place in the concentrated extract even when it was kept for a week at 4°. Both *A* and *B* did not give a positive reaction for glycosides.

Preparation of an Extract.—One kilogram of the coarsely powdered drug was soxhletted with 75% alcohol for eight hours and the extract was concentrated under reduced pressure to about 400 cc. and filtered. The filtrate was treated with neutral lead acetate 10% and lead subacetate 10% successively till all the tannins were precipitated. The extract was filtered, the precipitate was washed with distilled water, and the filtered washings were mixed with the extract. Excess of lead salt in solution in the mixed extract was precipitated by passing hydrogen sulfide gas and filtering off the precipitate. The excess hydrogen sulfide gas in the filtrate was removed by passing air through it and the filtrate was diluted with distilled water to make 1 liter. The straw-colored liquid gave positive tests for carbohydrates (4) and was used for pharmacological investigations and will henceforth be known as the "Prepared extract of gaozavan." One ml. of this extract represented one gram of the crude drug powder of gaozavan.

METHODS AND RESULTS

Cardiovascular System.—Arterial blood pressure was recorded from common carotid artery in dogs weighing 5 to 10 Kg. anesthetized with ether and followed by sodium barbital (100 mg./Kg.) intravenously. Table I shows the effect of the "Prepared extract of gaozavan." All injections were made through cannulated femoral vein.

TABLE I.—ACTION ON BLOOD PRESSURE

Dose of Prepared Extract, cc./Kg.	Average Maximum Fall in B. P., mm. Hg	Average Initial Pressure, mm. Hg
0.1	25	100
0.25	50	110
0.5	65	100

The heart rate fell in each case but came to normal within 5 to 15 minutes (Fig. 1). Venous pressure was simultaneously recorded but it did not reveal any significant change.

Action on Vasomotor Center.—The arterial pressure of the anesthetized dogs was recorded through the femoral artery. The two common carotid arteries were exposed and were clamped for half a minute each time and the rise in arterial pressure was recorded. Carotid occlusion was repeated two minutes after the administration of the prepared extract of gaozavan in a dose of 0.25 cc./Kg. through the contralateral femoral vein. There was no rise in the blood pressure. However, when the carotid occlusion was done again after another two minutes the rise in blood pressure was observed to a level slightly lower than the control (Fig. 2).

A dose of 0.25 cc./Kg. of the prepared extract caused a diminution in the force of contraction of the auricle and ventricle of intact heart in anesthetized dogs with open chest. The ventricle also showed signs of dilatation (Fig. 3).

A dose of 0.05 cc. caused slowing and diminution

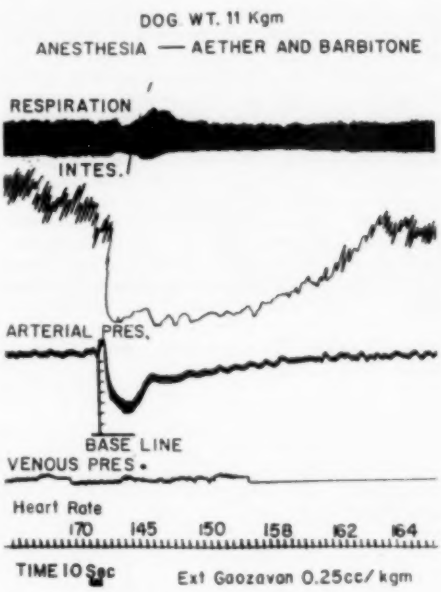

Fig. 1.—The tracings, reading from top to bottom, are: respiration, intact intestine, arterial blood pressure, and venous blood pressure.

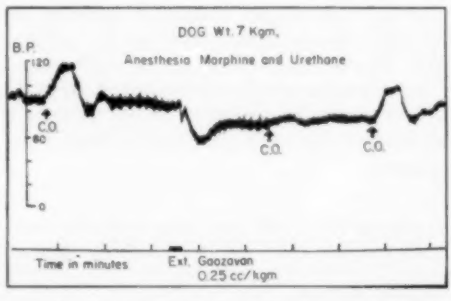

Fig. 2.—Action of the prepared extract on vasomotor center. At C. O. the carotids were clamped for half a minute.

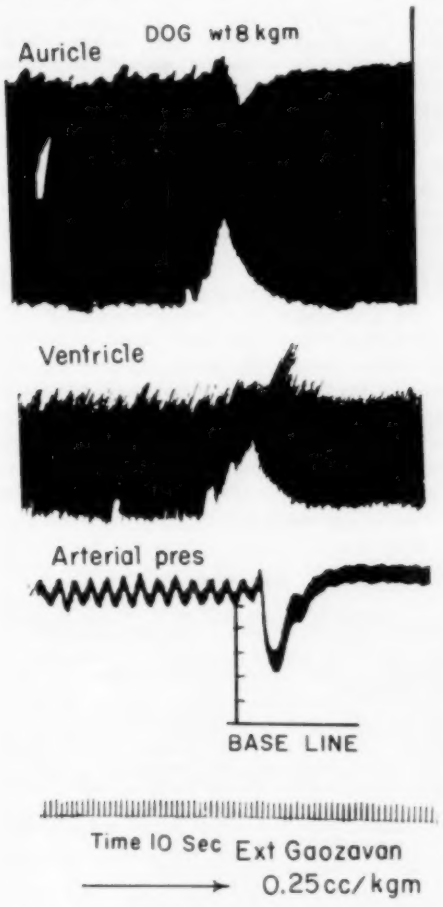

Fig. 3.—The effect of the extract on the auricle, ventricle, and arterial blood pressure of dog in intact heart. The dog had been anesthetised with ether and barbital sodium.

in the force of contraction in the isolated heart (Langendorff's method) (5) of rabbits weighing one to two Kg. A dose of 0.1 cc., however, stopped the heart in diastole but it recovered completely in about 15 minutes.

The blood vessels of the hind limbs of three dogs were perfused with oxygenated defibrinated blood of the same dog through the femoral artery and venous output from the femoral vein was measured. The results are given in Table II.

Blood vessels of the frog hind limbs were also perfused by the technique of Laewen-Trendelenburg (6). Flow of the frog Ringer fluid slowed down considerably after 0.05 cc. of the extract had been injected.

A needle connected to a perfusion bulb was inserted in the lateral ventricle through a drill hole made on the skull of anesthetized dog. A lumbar puncture needle was inserted in the cisterna magna. The perfusion was started when cerebrospinal fluid freely came out. One-half cc. to 5 cc. of the extract injected in the lateral ventricle did not cause any appreciable change either in the blood pressure recorded through the common carotid artery of the anesthetized animal or in the heart rate.

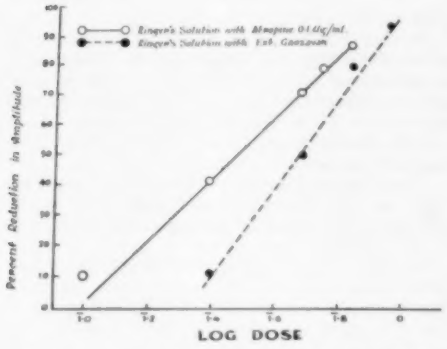

Fig. 4.—Comparison of spasmolytic activity. Graphs of log dose (abscissa) of atropine 0.1 μg./ml. and the prepared extract of gaozavan (abscissa) plotted against percentage reduction (ordinate) in the acetylcholine-produced spasm.

Action on Smooth Muscle.—The isolated strips of jejunum of rabbits weighing 1 to 2 Kg. were used. The strips were suspended in an isolated organ bath (7) and different doses of the prepared extract were added to the oxygenated Ringer solution contained in the inner bath of 50-cc. capacity. The effect of 0.4 μg. acetylcholine was observed before and after the addition of extract each time. The effect of gradually increasing doses (0.25 cc. to 1 cc.) was studied. The relaxation produced by 0.25 cc. was fairly appreciable. The spasmolytic activity of the extract was compared with atropine (0.1 μg./ml.). The results have been shown in the form of a graph (Fig. 4). It is obvious that the two drugs produce similar spasmolytic effect, atropine being effective comparatively in very small doses.

Experiments conducted on the isolated strips of intestine to determine the antispasmodic activity

TABLE II.—ACTION ON BLOOD VESSELS

No. Dog	Dog Wt., Kg.	Control Flow per min., cc.	Volume of Extract Injected into Femoral Artery, cc.	Flow Rate (in cc.) After 1 min.	2 min.	3 min.	4 min.	5 min.	6 min.
1	15	18	0.5	5	8	11	13	15	16
2	12	16	0.5	5	7	11	12	14	15
3	11	16	0.5	6	7	10	11	14	15

Fig. 5.—Antispasmodic activity. All contractions are due to acetylcholine 0.4 μg. added at the arrow. At G_1, G_2, G_3, and G_4 prepared extract of gaozavan 0.25 cc., 0.5 cc., 0.75 cc., and 1 cc. respectively was allowed to act for 30 seconds before the addition of acetylcholine.

revealed that 1 cc. of the extract almost completely prevented the induction of spasm by 0.4 μg. of acetylcholine (Fig. 5).

In dogs 6 to 12 Kg. anesthetized as before, the effect of the drug was investigated on the intestinal movements of the intact intestine by using both the balloon method and Jackson Enterograph. Doses as small as 0.1 cc./Kg. produced relaxation of the smooth muscle but in doses of 0.25 cc./Kg. the relaxation was marked (Fig. 1).

The prepared extract did not have any appreciable effect on the isolated uterus of guinea pigs in the concentrations employed in experiments with isolated intestine.

For studying the action on tracheal chain, young adult rabbits were killed by a blow on the head. The trachea was gently dissected out and sectioned into 12 rings of approximately the same width. Six to eight rings were connected by means of short loops of thread. The chain was then mounted in a 50-ml. bath containing Krebs-Ringer bicarbonate solution[1] maintained at 37.5° and continuously treated with 5% CO_2–95% O_2. The response to the drug was recorded with a light, sensitive lever which yielded a twelvefold magnification. The lever was balanced with the tracheal chain attached. The extract in a concentration 1 in 100 caused a mild relaxation of bronchial muscle.

Toxicity.—Acute toxicity was determined in mice. Doses as high as 5 cc./Kg. of the prepared extract given intravenously were tolerated by mice without any evidence of toxic symptoms. No effort was made to determine the LD_{50}.

[1] The Krebs-Ringer bicarbonate solution had the following composition, in milliequivalents per liter: Na, 137.4; K, 5.79; Ca, 4.93; Mg, 2.32; Cl, 125.1; HCO_3, 22.0; SO_4, 2.32; H_2PO_4, 1.16; glucose, 0.048 (molar).

SUMMARY

A dealcoholized, detannated extract of *Onosma bracteatum* lowers the blood pressure and depresses the heart and causes vasoconstriction. It has a direct depressant action on the cardiac muscle. It relaxes the small intestine and prevents the stimulating action of acetylcholine on the intestine. The drug appears to be useful as a spasmolytic.

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Cordyceps sinensis (Berkeley) Saccardo: Structure of Cordycepic Acid*

By R. CHATTERJEE, K. S. SRINIVASAN,† and P. C. MAITI

The botanical character of the fungus *Cordyceps sinensis* (Berkeley) Saccardo has been described. It has yielded cordycepic acid, $C_7H_{12}O_8$, whose structure has been elucidated to be 1,3,4,5-tetrahydroxy-cyclohexanoic acid, an isomer of quinic acid.

CORDYCEPS is an interesting genus of fungus for its characteristic parasitic habit on larvae and pupae of insects, and even on full grown insects. Several species are known from the Australasian region, some of the largest known specimens originating from Australia proper. *Cordyceps sinensis* (Berkeley) Saccardo (1) has long been regarded as a celebrated drug in the Chinese Pharmacopeia. It is known under different local names such as *Hia Tsao Tom Tchom, Hia Tsao Tong Tchong, Hea Tsaom*

* Received June 21, 1956, from the Department of Chemistry, Medical College, Calcutta, India.
† Curator, Industrial Section, Indian Museum, Calcutta, India.
Our thanks are due to the British Museum for the determination of the specimen of the fungus, and to Dr. C. V. Subrahmaniam, Reader in Botany, Madras University, for help in old botanical literature.
Abstracted from a part of a thesis submitted to the University of Calcutta, by P. C. Maiti in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Taong Chung, Hea Tsao Taog Chung, Tung Chung Chow, and *Yartsa Gunbu;* the several names meaning *worm in winter and grass in summer*. It is also considered as a delicacy by the Chinese and is served to guests in a powdered form stuffed into duck's stomach. In this form, it is called as *Tsong Tsao Yah Dz*, which in Mandarin means *winter worm duck*.

The medicinal properties of this fungus have been briefly referred to by various earlier authors. Berkeley (2) mentions it as a restorative. Gist Gee (3) states that it is used only as a tonic, and according to the Chinese, the grass-like growth from the top of the head is a tonic for the upper part of the body, and that the body of the worm is a tonic for the lower part of the body. It is considered to be valuable in the cure of tuberculosis, in dissolving phlegm, and in the treatment of kidney disorders (4). Brewster and Alsberg (5) recorded that an aqueous solution of the fungus growing on top of the caterpillar was acidic and the neutral solution was toxic.

moderately hard and rigid but easily breaking with a white fracture. Except for the outer skin of the caterpillar, which is seen intact, the portion of the caterpillar is seen filled up with a sort of a white pithy substance which is found to be made up of a compact mass of fungal filaments themselves. The "stem" or the portion which grows out from the anterior part of the caterpillar is, likewise, somewhat hard and brittle when dry. In its natural condition, it is cylindrical. In mature specimens, this broadens out above into the fertile portion bearing the perithecia in large numbers (Figs. 1f–1g). The apex of the "stem" is somewhat broadly pointed (Fig. 1g). The color of the fungal portion above is dark, and in the early stages, the stem is solid, although in later stages, it tends to become somewhat hollow.

Usually a single stem grows out of the host (Figs. 1a-1e). But occasionally, two perfect ones are seen developed in one specimen, one quite independent of the other (Figs. 1f and 2a). Such a condition does not appear to have been recorded for this species earlier. Berkeley (2) recorded that the head of the fungus is sometimes split into two or three linear portions (*cf.* his illustrations also on Tab. VIII, Fig. 1). The condition recorded by Berkeley, however, is entirely different from the one obtained in specimens by the present authors.

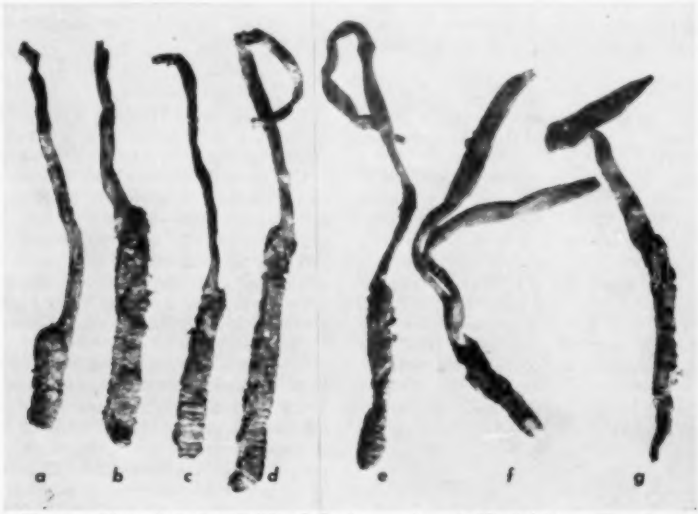


Fig. 1.—(Actual size). Habit of *Cordyceps sinensis* (Berk.) Sacc. Figs. 1a–1e, with sterile stems only, Figs. 1f–1g, with fertile portions, Fig. 1f showing 2 fertile stems in one specimen.

CHARACTERISTICS

The fungus grows out as a parasite from the anterior part of the caterpillar of a Lepidopteran Moth (Figs. 1a–1g and 2a). With the available specimens, it was not possible to determine exactly to which species the host belongs, as most of the features of diagnostic value were found either greatly distorted or lost. The lower caterpillar portion, in the dry state is dark brown in color outside,

The perithecia are developed superficially on the fertile portion of the fungus. They are generally free, but by the crowded growth of the perithecia, their sides become closely adpressed in some cases (Fig. 3). The perithecia are more or less flask-shaped, but their shapes vary considerably, sometimes appearing more linear. The basal part of the perithecium is broad, and the ostiole is of moderate size. The specimens examined by the present authors contained in most cases, only the sterile

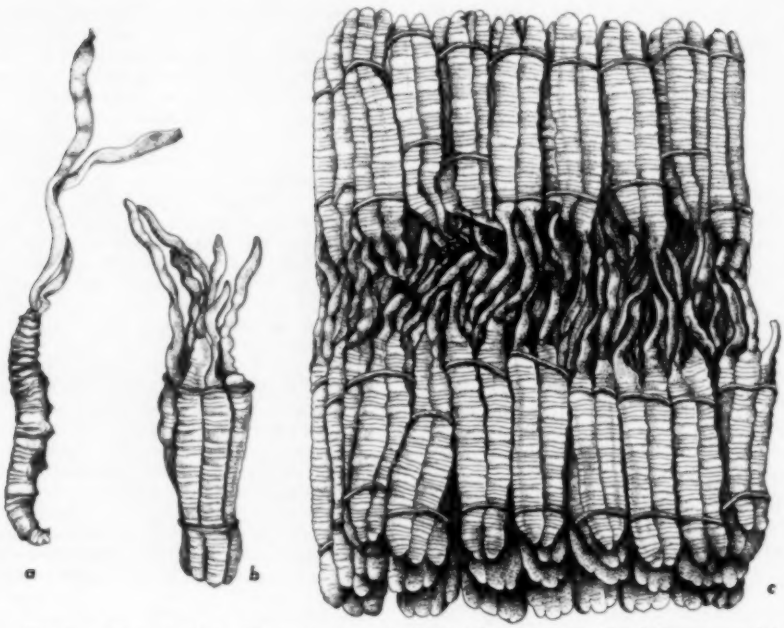


Fig. 2a.—(Actual size). *Cordyceps sinensis* (Berk.) Sacc. Fig. 2b (Semi-diagrammatic). A small bundle of 6–10 specimens tied with a fine thread. Fig. 2c (Semi-diagrammatic). A packet showing the arrangement of the bundles of specimens.

stem portions. In the few cases where the fertile portions were seen, it was found that the specimens were immature.

The following are some of the measurements of the fungus examined: The lower caterpillar portion measures from 32 to 42 mm. long, and 4 to 5 mm. thick; the sterile stem portion is 10 to 15 mm. long and 2 mm. thick; the fertile portion measures from 17 to 25 mm. long, and 3 to 4 mm. thick; the apical portion measures about 1.5 mm. long; the perithecia measure from 165 μ to 230 μ long, and at the base and at its broadest part, 65 μ to 150 μ across; the ostiole is from 16 μ to 33 μ wide; the asci were found to be in very early stages of development, and about 33 to 50 μ long and 3 to 5 μ broad. No mature ascospores could be seen in the materials examined.

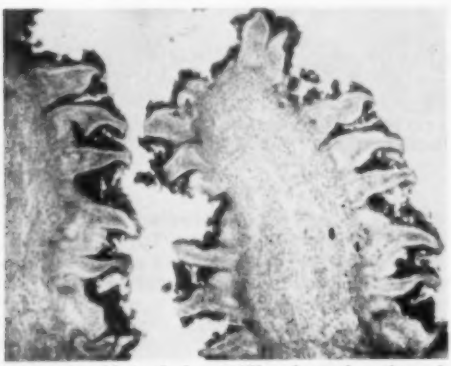


Fig. 3.—(Actual size ×45). A section through the fertile portion (*vide* Figs. 1f and 1g), showing the distribution of perithecia.

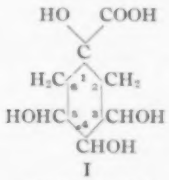
The drug as it comes to the market is seen in small packets of about 56 bundles of specimens, each bundle tied with fine threads. The bundles contain about 6 to 10 specimens each. Figures 2b and 2c illustrate the arrangements of the specimens and the bundles, respectively. It is understood that materials of best medicinal value originate from Szechwan and Kansu Provinces in N. China.

Cordyceps sinensis is imported to Calcutta in a limited quantity at particular seasons of the year. Fresh materials for the present investigation were obtained locally from a Chinese dealer. Some specimens were also obtained from Tibet by courtesy of the Indian Ambassador at Lhasa.

Despite the medicinal attributes of this drug, nothing is known so far, of its chemistry. It is, therefore, desirable to have a chemical investigation of this species in order to appreciate fully its therapeutic value. An antibiotic substance cordycepin, $C_{10}H_{13}O_3N_5$, has been isolated from another species, *C. militaris* (6). *C. sinensis* has been found, however, to yield an acid, $C_7H_{12}O_6$, which has been named cordycepic acid and has been proved to be an isomer of quinic acid (7).

The well-powdered, defatted, whole fungus yields a good quantity (7%) of colorless, transparent, highly crystalline cordycepic acid, which is optically active, sweet to taste and acidic to litmus. It forms an acetone compound, and responds to Molisch's test, but is inert to Fehling's solution.

Cordycepic acid is a tetrahydroxy monobasic acid; it forms a crystalline tetra-acetate, a tetra-benzoate, and a mono-methyl ester. It does not form a lactone. One molecule of it takes up two molecules of periodic acid indicating as one of the possibilities the presence of 1,2,3-trihydroxy system in its molecule. The periodic acid oxidation product yields on further oxidation with bromine, citric acid. All these data point cordycepic acid (I) to be an isomer of quinic acid (Table I).



I

Cordiceyic acid

Tetra-acetyl Cordycepic Acid.—Two-tenth gram of the acid was in one case refluxed with acetic anhydride for three hours, and in a second case with acetic anhydride and a few drops of pyridine; the mixture was diluted with water and kept in a refrigerator. An oil separated first, which set to a crystalline mass and could be purified by repeated crystallization from aqueous ethanol, m. p. 126°, $[\alpha]_D^{28}$ + 29.2°(EtOH).

Anal. Calcd. for $C_{18}H_{20}O_{11}$: C, 50.0; H, 5.55; tetra-acetyl value, 47.7 Found: C, 50.03; H, 5.86; acetyl value, 49.8.

Tetrabenzoyl Cordycepic Acid.—Two-tenth gram of the acid was kept cold for 24 hours with benzoyl chloride (5 cc.) and pyridine (5 cc.), and then the mixture was treated with ice cold water. The crude benzoate which separated initially as an oil, solidified on standing and then was filtered. The residue was boiled with water to free it from benzoic acid and finally purified by repeated crystallization from chloroform-alcohol-water mixture, m.p. 151°. It retained one molecule of water of crystallization even on drying *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $C_{35}H_{28}O_{10}$, H_2O: C, 67.09; H, 4.79. Found: C, 67.55; H, 5.23.

TABLE I.—A COMPARISON OF THE PROPERTIES OF CORDYCEPIC AND QUINIC ACIDS

Properties Molecular formula M. p.	Cordycepic acid $C_7H_{12}O_6$ 168°	Quinic acid $C_7H_{12}O_6$ 162°
Sp. rotation	$[\alpha]_D^{28}$ + 6.8°(H_2O)	$[\alpha]_D^{28}$ − 42.1°(H_2O)
Functional Group	4OH-, 1-COOH group	4OH-, 1-COOH group
Derivatives	Tetra-acetate, m. p. 126°, $[\alpha]_D^{28}$ + 29.5° (EtOH)	Tetra-acetate, m. p., 132–136°, $[\alpha]_D^{28}$ − 22.5° (EtOH)
	Tetrabenzoate, m. p., 151°	Tetrabenzoate, m. p., 137–138°
	Acetone compound of the free acid, m. p., 56°	Acetone compound of the γ-lactone, m. p., 140–141°
	No lactone	γ-lactone, m. p., 187°

The molecular model after Stuart and Briegleb indicates quinic acid to be of *cis*-structure, where the carboxyl group in position -1 is *cis*- with the hydroxyl group in either of the positions -3 and -5. Cordycepic acid may be said to differ in this point from quinic acid, its carboxyl group being *trans*- to either of the hydroxyl groups in -3 and -5, for it does not form a lactone.

The experimental data, *viz.*, response to Molisch's test, formation of acetone compound of the acid as well as of the methyl ester, indicate that the hydroxyl groups located at 3, 4, and 5 are in all probability *cis*- with respect to each other and *trans*- with respect to the carboxyl group at -1 of the acid.

EXPERIMENTAL

Isolation of Cordycepic Acid.—Well-ground, air-dried fungus (80 Gm.) was soxhletted successively with petroleum ether (60–80°) and ethanol (95%). The petroleum ether extract yielded a brown oil which showed the presence of sterols. The ethanolic extract yielded 5.6 Gm. of colorless needles of cordycepic acid, m. p. 168°, $[\alpha]_D^{28}$ + 6.8°(H_2O). The acid is extremely soluble in water, sparingly soluble in absolute ethanol and other organic solvents.

Anal. Calcd. for $C_7H_{12}O_6$: C, 43.75; H, 6.25, mol. wt. 192. Found: C, 43.81; H, 6.18, mol. wt. 207 (titrimetric), showing monobasicity of the acid.

Acetonyl Derivative of Cordycepic Acid.—Fifty milligrams of the acid was suspended in dry acetone (75 cc.) and dry hydrochloric acid gas was passed into the mixture with constant shaking till the acid dissolved. The solution was then allowed to stand for 48 hours and neutralized with silver carbonate (Congo red), and filtered. The syrupy residue left on evaporation of the filtrate was crystallized from petroleum ether (40–60°) to yield fine needles of the mono-acetonyl derivative of the acid, m. p. 56°.

Anal.—Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.89. Found: C, 51.73; H, 6.7.

Acetonyl Derivative of Methyl Cordycepate.—The acetonyl derivative of the acid could be esterified smoothly with diazomethane under the usual condition. The ester was purified by repeated crystallization from benzene, m. p. 132°.

Anal. Calcd. for $C_{11}H_{18}O_6$: C, 53,67; H, 7.31. Found: C, 53.54; H, 7.48.

Cordycepic Acid Esters.—The acid did not dissolve in ether or benzene. All attempts to react the acid in suspension with diazomethane were abortive. The sodium or the silver salt of the acid did not react successfully with alkyl halides.

The acetonyl derivative of the acid, however, reacted readily with diazomethane, because of its solubility in ether.

Attempted Formation of Cordycepic Acid Lactone.—All attempts to prepare cordycepic acid lactone under usual conditions failed. In one instance, 0.2 Gm. of the acid was heated with concen-

trated hydrochloric acid for ten hours. Unchanged acid was recovered from the reaction mixture.

The acid (0.2 Gm.) was obtained unchanged after heating with 20% sulfuric acid for four hours or after heating with 20 cc. of methanol saturated with hydrochloric acid gas.

Oxidation Experiments: Isolation of Citric Acid.—Each 0.1174 Gm. of the acid consumed 2.4 cc. (0.54 *M*) periodic acid in twenty-four hours at 25.8°. The value corresponds to 2.12 molecules of periodic acid per molecule of cordycepic acid.

The mixture was kept at room temperature for two days. It was treated with silver carbonate and the precipitate was filtered. The filtrate was kept overnight with one drop of bromine, and then treated with dilute sodium hydroxide solution till the mixture was pale brown, and boiled for one hour. The mixture was then treated with acetic acid and boiled with calcium chloride solution. The white precipitate was filtered and then decomposed with dilute sulfuric acid and filtered and the filtrate was extracted with ethyl acetate. The crystalline acid, m. p. 153°, obtained on removal of the solvent was identified to be citric acid and it did not depress the m. p. of an authentic sample of citric acid.

SUMMARY

1. The fungus *Cordyceps sinensis* (Berkeley) Saccardo grows out as a parasite from the anterior part of the caterpillar of a Lepidopteran Moth. Usually a single stem and rarely two, grows out of the host. The perithecia are developed superficially on the fertile portion of the fungus.

2. An acid, which has been named cordycepic acid, $C_7H_{12}O_8$, has been isolated in 7% yield from the fungus. Its structure has been determined as 1,3,4,5-tetrahydroxy-cyclohexanoic acid, isomeric with quinic acid. It differs mainly from the natural quinic acid in being dextrorotatory and not forming the lactone.

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Anticonvulsants: Spirohydantoins and Derivatives of Tetrahydronaphthalene*

By JOHN A. FAUST, LEONARD H. JULES,† LIM YEE, and MELVILLE SAHYUN

The spirohydantoin derived from 2-tetralone was prepared and found to be a nontoxic compound which possessed promising anticonvulsant potency in both electroshock and metrazol-induced spasms in the experimental animal. Congeners of this spiro-compound and other derivatives of tetrahydronaphthalene were synthesized for comparative biologic activity, but none exhibited greater potency than the parent compound.

SINCE THE DISCOVERY that 5,5-diphenylhydantoin (1) is an effective drug in the treatment of grand mal epilepsy, numerous hydantoin derivatives have been prepared and tested as potential antiepileptic agents. Spirohydantoins, those derived from cyclic ketones, have been included in many of these studies (2–5), but these compounds, with the exception of 9-fluorenone hydantoin which has been studied clinically (6), have apparently elicited little interest as possible agents for the treatment of epilepsy in human subjects.

The hydantoin derived from 3,4-dihydro-1-(2H)-naphthalenone (1-tetralone) was first described by Novelli (7) who with his co-workers (8), reported that it was without hypnotic activity. Later it was tested as an anticonvulsant in the cat, and its potency was reported to be of a relatively low order (5).

During an investigation on potential anticonvulsant agents, we prepared the hitherto unknown hydantoin[1] of 3,4-dihydro-2-(1H)-naphthalenone (2-tetralone), which on testing was found to possess high activity and low toxicity in the experimental animal. Accordingly, a number of substituted derivatives of

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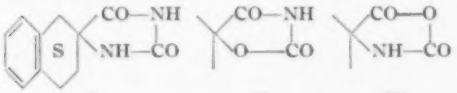
† Present address: The Purex Corporation, South Gate, Calif.

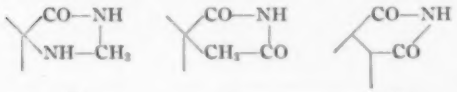
[1] Since the completion of this work, this compound, its N-methyl and N,N'-dimethyl derivatives have been reported in the literature: Novelli, A., *Anales farm. y bioquim. (Buenos Aires)* **2 21**, 81(1954); *Chem. Abst.*, **50**, 4922(1956).

this parent compound, as listed in Table I, were synthesized in order to study the effect of the various substituents on the physiological response. This study was extended to include hydantoins of ketones whose structures are similar to 2-tetralone, and these are listed in Table II. It will be noted that most of them are spirohydantoins in which the spiro carbon atom is separated from an aromatic system by a methylene group, an arrangement found in the hydantoin of 2-tetralone.

The isomeric spirohydantoins derived from 1- and 2-tetralone may be regarded as spiro systems in which hydantoin rings and tetrahydronaphthalene rings share a mutual carbon atom. The physiologic activities of these isomers are considerably different: the hydantoin of 2-tetralone (I) is much more potent and



I II III



IV V VI

less toxic than its isomer. It was therefore considered of interest to incorporate the tetrahydro-2-naphthyl group found in the more active isomer into structures other than hydantoins in the hope that the products would be active anticonvulsants. Thus, since certain oxazolidine-dione derivatives, such as Tridione®, are useful in the treatment of idiopathic epilepsy, a tetrahydro-2-naphthyl oxazolidinedione (II) and the isomeric dione (III), in which the —NH— and —O— groups of the oxazolidine structure are interchanged, were prepared. Similarly, a tetrahydro-2-naphthyltetrahydroglyoxaline-4-one (IV) was synthesized as a representative counterpart of 5,5 - diphenyltetrahydroglyoxaline - 4 - one recently reported to have definite anticonvulsant potency (9). Since derivatives of succinimide have recently come into prominence in the treatment of epilepsy (10, 11), the synthesis of two tetrahydronaphthyl succinimides (V, VI) was also made a part of the present investigation.

Pharmacology.—All of the compounds reported herein were investigated for their anticonvulsant activity at the Department of Pharmacology of Cutter Laboratories under the direction of Dr. V. P. Seeberg. The detailed results of these pharmacological studies are to be published elsewhere. The activities for the various compounds as obtained by the Maximal Electroshock Seizure Test and the approximate toxicities as determined in mice are given in the tables. It is obvious from these data that the hydantoin of 2-tetralone (Compound S.2–676) and its 2-thio analog (Compound S.2–846) showed the greatest potencies in this series. However, since the toxicity of the thiohydantoin was approximately ten times greater than that of the hydantoin itself, its therapeutic index was less favorable.

METHODS OF PREPARATION

The unsubstituted hydantoins were prepared from the various ketones by the Bucherer reaction (12). The 3-alkyl derivatives listed in Table I were readily obtained by the alkylation of the parent hydantoin, as is exemplified by the preparation of the 3-ethyl derivative in the Experimental. Contrary to our experience with the 3-alkyl derivatives, considerable difficulty was encountered in the synthesis of an example of a 1-alkyl derivative. The procedure employed was one described previously (13) in which a ketone was reacted with a primary amine to form a substituted tetrahydronaphthylidenimine which was treated without isolation with hydrogen cyanide to produce an alkyl aminonitrile. Direct interaction of the crude aminonitrile with potassium cyanate and hydrochloric acid completed the synthesis of the 1-alkyl hydantoin. By this series of reactions, there was obtained a low yield of crystalline material whose properties and analysis were consistent with those expected for 1-butyl-7,8-benzo-1,3-diazospiro-[4.5]decane-2,4-dione (Compound S.2-1046).

The one example of a 2-thiohydantoin (Compound S.2-846) was prepared by the reaction of the appropriate amino acid and thiourea, a process heretofore reported (14). A 2,4-dithiohydantoin (Compound S.2-816) was obtained from the corresponding cyanohydrin by reaction with carbon disulfide and ammonia (15). Desulfurization (16) of the 2-thiohydantoin with Raney nickel in refluxing ethanol provided an efficient means of preparing the tetrahydronaphthyltetrahydroglyoxaline - 4 - one (Compound S.2-913).

With the exception of 1-allyl-2-tetralone, which was prepared by the alkylation of 2-tetralone (17), all of the ketones employed as intermediates in this series were known compounds. Literature references to these ketones are given in the footnotes of the tables in which they appear. One of these ketones, octahydro-2(1H)-naphthalenone(2-decalone), exists in two forms, *cis* (18) and *trans* (19). In the present work, 2-decalone was prepared by the oxidation of 2-decalol according to the procedure described in the preparation of menthone (20). The intermediate 2-decalol was obtained by the alkaline hydrolysis of a commercial sample of decahydro-2-naphthyl acetate.[2] The properties of the 2-decalone so obtained were in agreement with those reported in the literature (21), and it was evidently a mixture of the *cis* and *trans* forms, since two hydantoins, Compounds S.2-836 A & B, were obtained from it. The configurations of these hydantoins were not established; however, it is interesting to note that

[2] Aldrich Chemical Company, Milwaukee, Wis.

TABLE I

Code No. S.2-	R	R′	R″	R‴	M.p., °C.	Yield, %	Formula	Nitrogen, %, Calcd.	Nitrogen, %, Found	Approximate PD_{50}[a] in mg./Kg.	Approximate LD_{50} in mg./Kg. Intraperitoneally in Mice
676	H	H	H	H	267–268	84	$C_{14}H_{12}N_2O_2$	12.91	12.85	50	2000
700	H	CH_3	H	H	181–182	30	$C_{15}H_{14}N_2O_2$	12.17	12.00	100	500
870	H	C_2H_5	H	H	163–164	46	$C_{16}H_{16}N_2O_2$	11.46	11.23	100	400
827	H	C_4H_9	H	H	136.5–137.5	45	$C_{18}H_{20}N_2O_2$	10.29	10.29	300	800
961	H	$CH_2(CH_2)_4CH_3$	H	H	130–131	37	$C_{20}H_{24}N_2O_2$	9.33	9.40	800	800
1046	C_4H_9	H	H	H	116–117	4	$C_{18}H_{20}N_2O_2$	10.29	9.93	175	200
871	H	CH_2CH_2OH	H	H	166–168	69	$C_{15}H_{14}N_2O_3$	10.76	10.77	100	700
792	H	H	H	CH_3O[b]	263–264	70	$C_{15}H_{14}N_2O_3$	11.38	11.35	900	4000
798	H	H	$CH_2CH{-}CH_2$	H	181–183	33	$C_{16}H_{16}N_2O_2$	10.93	10.73	100	750
846 (2-thio)	H	H	H	H	203–204.5	27	$C_{15}H_{14}N_2OS$	12.06	11.99	35	200
816 (2,4-dithio)	H	H	H	H	208–209	9	$C_{15}H_{14}N_2S_2$	11.28	11.19	300	>800

[a] PD_{50} indicates the approximate dose in mg./Kg. intraperitoneally to protect 50% of the mice against seizures in the maximal electroshock seizure test. [b] The intermediate ketone, 3,4-dihydro-7-methoxy-2-(1H)-naphthalenone, was prepared according to Soffer, M. D., Cavagnol, J. C., and Gellerson, H. E., *J. Am. Chem. Soc.*, **71**, 3857(1949). Compounds S.2-676 and S.2-792 were recrystallized from acetic acid; the others were recrystallized from either isopropyl alcohol or dilute isopropyl alcohol.

their anticonvulsant activities, both against spasm induced by electroshock and by metrazol, and their toxicities were demonstrably different. The lower melting form (Compound S.2-836 B) was both more active and more toxic than the higher melting form (Compound S.2-836 A).

Two hydantoins (Compounds S.2-790 A & B) were also obtained from methyl 1,2,3,4-tetrahydro-1-naphthyl ketone, and these were ostensibly the diastereoisomeric forms arising from the two asymmetric carbon atoms: one at the 5-position of the hydantoin, the other in the 1-position of the tetrahydronaphthyl ring. These compounds differed in their physiological, as well as in their chemical properties. Again, the lower melting form (Compound S.2-790 A) was considerably more effective in providing protection against both electrically-induced and chemically-induced seizures in animals. This low melting form was also somewhat more toxic than the higher melting compound.

2 - Hydroxy - 1,2,3,4 - tetrahydro - 2 - naphthonitrile, obtained from 2-tetralone and hydrogen cyanide, served as a convenient intermediate for the preparation of the oxazolidine-dione derivative (Compound S.2-733). Alcoholysis of the cyanohydrin yielded ethyl 2-hydroxy-1,2,3,4-tetrahydro-2-naphthoate which, when treated with urea in the presence of sodium ethylate, was transformed to the desired oxazolidine-dione. The isomeric dione, Compound S.2-840, was prepared by the cyclization of the compound formed when 2-amino-1,2,3,4 - tetrahydro - 2 - naphthoic acid was allowed to react with ethyl chlorocarbonate in an alkaline medium. The cyclization was effected by means of warm thionyl chloride.

The succinimide derivatives (V, VI) were prepared by heating the ammonium or amine salts of the corresponding dicarboxylic acids. One of the dicarboxylic acids is a known compound (22); the other was prepared by a series of reactions (10) in which 2-tetralone was first condensed with ethyl cyanoacetate to form a cyano-naphthylidine acetate. The addition of potassium cyanide to the naphthylidine ester resulted in a dicyano compound which upon hydrolysis and decarboxylation yielded the desired 2-carboxy-1,2,3,4-tetrahydro-2-naphthalene acetic acid.[3]

EXPERIMENTAL[4]

7,8 - Benzo - 1,3-diazaspiro[4.5]decane - 2,4-dione (Compound S.2-676).—While stirring, a mixture of 154 Gm. (1.05 mole) of 2-tetralone (23), 91 Gm. (1.4

[3] The analytical data were obtained by R. H. McCutcheon of these laboratories.

[4] All melting points are corrected.

TABLE II.—HYDANTOINS OF KETONES

Code No. S.2-	Ketone	M. p. °C.	Yield, %	Formula	Nitrogen, % Caled.	Nitrogen, % Found	Approximate PD_{50}[a] in mg./Kg.	Approximate LD_{50} in mg./Kg. Intraperitoneally in Mice
712	2-Indanone[b]	255–256	71	$C_{11}H_{10}N_2O_2$	13.96	13.83	100	500
705	7-Acenaphthone[c]	277–279	53	$C_{14}H_{10}N_2O_2$	11.76	11.61	200	800
836 A	Octahydro-2(1H)-naphthalenone[d]	288–293	65[e]	$C_{12}H_{18}N_2O_2$	12.60	12.49	800	>800
836 B		259–262	20[e]	$C_{12}H_{18}N_2O_2$	12.60	12.56	500	800
740	2-Benzylcyclohexanone[f]	251–252	50	$C_{13}H_{16}N_2O_2$	10.85	10.82	none	>800
747	1,4-Diphenyl-2-butanone[g]	243–244	76	$C_{18}H_{18}N_2O_2$	9.52	9.55	none	>800
790 A	Methyl 1,2,3,4-tetrahydro-1-naphthyl ketone[h]	184–186	60	.....	11.47	11.40	300	600
790 B[k]		259–261	60	$C_{14}H_{16}N_2O_2$	11.47	11.45	none	>800
809	2,3-Dihydro-4(1H)-quinolone[i]	266–267	42	$C_{11}H_{11}N_2O_2$	18.99	18.84	800	>800
765[k]	3-Chromanone[j]	288–289	18.6	$C_{11}H_{10}N_2O_2$	12.84	12.59	100	800

[a] PD_{50} indicates the approximate dose in mg./Kg. intraperitoneally to protect 50% of the mice against seizures in the maximal electroshock seizure test.
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[k] Compounds S.2-790B and S.2-765 were recrystallized from acetic acid. Compound S.2-790A from chloroform-heptane, Compound S.2-705 from dilute acetone, and Compound S.2-809 from ethanol-heptane. The others were recrystallized from either isopropyl alcohol or dilute isopropyl alcohol.

mole) of potassium cyanide, 269 Gm. (2.8 mole) of ammonium carbonate, and 1200 cc. of isopropyl alcohol was heated to 55–60°, and this temperature was maintained for six hours. The cooled mixture was filtered, and the finely divided solid was dissolved in dilute sodium hydroxide. After the solution had been treated with decolorizing charcoal, it was filtered and acidified. The solid which separated was collected on a filter, washed with water and dried. Yield, 191 Gm. (84%); m. p. 267–268° after recrystallization from glacial acetic acid.

7,8 - Benzo - 3 - ethyl - 1,3 - diazaspiro[4.5]decane-2,4-dione (Compound S.2-870).—A solution of 10.8 Gm. (0.05 mole) of the hydantoin (Compound S.2-676) in 25 cc. of water containing 3.3 Gm. (0.05 mole) of potassium hydroxide was combined with a solution of 5.5 Gm. (0.05 mole) of ethyl bromide in 50 cc. of methanol. The homogeneous mixture was refluxed for three hours, cooled, and the solid which separated was recrystallized from isopropyl alcohol. Yield, 5.5 Gm. (46%); m. p. 163–164°.

Other 3-N-substituted hydantoins (Compounds S.2-700, 827, and 871) were prepared in a similar fashion using the appropriate alkyl halide or sulfate as the alkylating agent.

1 - Butyl - 7,8 - benzo - 1,3 - diazaspiro[4.5]-decane - 2,4 - dione (Compound S.2 - 1046).—A slight exothermic reaction occurred when 7.3 Gm. (0.1 mole) of *n*-butylamine was added gradually to 14.6 Gm. (0.1 mole) of 2-tetralone, and the mixture was warmed on a water bath for one hour. It was cooled and added to a cold, stirred mixture of 40 cc. of 75% aqueous acetic acid and 2.7 Gm. (0.055 mole) of sodium cyanide at such a rate that the temperature remained below 10°. An additional 2.7 Gm. of sodium cyanide was added, the mixture was cooled to 0°, and 8.9 Gm. (0.11 mole) of potassium cyanate was added. After the mixture had been at 70° for 15 minutes, 29 cc. of concentrated hydrochloric acid was added, and heating at 95–100° was continued for one hour. The cooled dark mixture was diluted with water and extracted with ether. The ether solution was washed with sodium bisulfite and then extracted with dilute sodium hydroxide. Acidification of the alkaline aqueous extract precipitated an oil which partially solidified, and was purified by dissolving in dilute sodium hydroxide, treating with charcoal and reprecipitating with acid. Several recrystallizations from dilute isopropyl alcohol yielded 1 Gm. (4%) of colorless needles melting at 116–117°.

1 - Allyl - 3,4 - dihydro - 2(1H) - naphthalenone.—A solution containing 12.5 Gm. (0.085 mole) of 2-tetralone and 7.6 Gm. (0.1 mole) of allyl chloride in

TABLE III.—ADDITIONAL PHARMACOLOGIC DATA[a]

Code No. S.2-	Approximate PD_{50}[b] in mg./Kg.	Approximate LD_{50} in mg./Kg. Intraperitoneally in Mice
733	100	600
771	150	600
787	100	500
823	400	400
840	none	100
913	500	800

[a] These data are for those compounds which are described in the text but are not included in Tables I and II.
[b] PD_{50} indicates the approximate dose in mg./Kg. intraperitoneally to protect 50% of the mice against seizures in the maximal electroshock seizure test.

45 cc. of dry ether was added during 15 minutes to a stirred suspension of 3.9 Gm. (0.1 mole) of sodium amide in 55 cc. of dry ether. The dark mixture was stirred under reflux overnight (16 hours), cooled, and water was added. The layers were separated, and the ether layer was dried and distilled. Yield, 13.8 Gm. (87%); b. p. 95–98° (0.3 mm.). The hydantoin derived from this ketone is Compound S.2-798.

2 - Amino - 1,2,3,4 - tetrahydro - 2 - naphthoic Acid (Method A).—A mixture of 21.6 Gm. (0.1 mole) of the hydantoin of 2-tetralone (Compound S.2-676), 50 cc. of approximately 40% sodium hydroxide and 150 cc. of propylene glycol was refluxed for 24 hours and poured into 300 cc. of water. The solution was treated with charcoal, filtered, acidified, and refiltered. The filtrate was neutralized to *p*H 5–6, and the amino acid which separated was isolated and purified by treatment with charcoal in acid solution and reprecipitated by neutralization to *p*H 5–6 with sodium bicarbonate. The amino acid was obtained as a mono-hydrate; yield, 16.6 Gm. (79%); m. p. 300–302° (closed tube). It is insoluble in water and in common organic solvents.

Potentiometric titration showed only one inflection point at the isoelectric point which is about *p*H 6. Formol titration showed an inflection point at about *p*H 7.5.

Anal.—Calcd. for $C_{11}H_{13}NO_2.H_2O$: N, 6.70. Neut. equiv. 209. Found: N, 6.69. Neut. equiv. 203.

(Method B).—To a stirred, cooled mixture of 50 Gm. (1 mole) of sodium cyanide, 59 Gm. (1.1 mole) of ammonium chloride, 69 cc. (1 mole) of 28% ammonium hydroxide and 240 cc. of water was added a solution of 146 Gm. (1 mole) of 2-tetralone in 160 cc. of methanol. The flask was stoppered and heated at 55–60° for 16 hours with occasional agitation. The heterogeneous reaction mixture, consisting of two liquid layers, was poured into 800 cc. of cold 36% hydrochloric acid, and after saturating with hydrogen chloride, the heterogeneous mixture was allowed to remain at room temperature overnight. It was then refluxed for six hours, whereupon a large volume of gas was evolved. The mixture was diluted with a liter of water, steam distilled to remove some oily impurities, and diluted with water to a volume of about 3 liters. Charcoal was added, the mixture was filtered, and the filtrate was neutralized with ammonia to *p*H 6. The solid which separated was purified by treatment with charcoal in acid solution and reprecipitating as before. Yield, 162 Gm. (78%); m. p. 298–299° (closed tube).

7,8 - Benzo - 1,3 - diazaspiro[4.5]decane - 4 - one-2 - thione (Compound S.2 - 846).—A mixture of 15.7 Gm. (0.075 mole) of 2-amino-1,2,3,4-tetrahydro-2-naphthoic acid monohydrate and 22.8 Gm. (0.3 mole) of thiourea was heated at 200–210° for three hours, during which it liquefied, and ammonia was evolved. The cooled residue was stirred in cold dilute hydrochloric acid until a solid was obtained. The solid was isolated, dissolved in warm, dilute sodium hydroxide, and treated with decolorizing charcoal. Acidification of the solution precipitated a solid which was recrystallized from dilute isopropyl alcohol. Yield, 4.8 Gm. (27%); m. p. 203–204°.

2 - Hydroxy - 1,2,3,4 - tetrahydro - 2 - naphthonitrile.—A slurry, prepared from 55.1 Gm. (0.22 mole) of the bisulfite addition product of 2-tetralone (24), 150 cc. water and 75 cc. ether, was stirred at 5–10° while a solution of 29.4 Gm. (0.6 mole) of sodium cyanide in 60 cc. of water was added over a 10-minute period. After the mixture had been stirred for three hours at room temperature, the layers were separated, the aqueous layer was extracted with ether, and the combined ether solutions were extracted with dilute sodium bisulfite. Evaporation of the dried ether solution left an oily residue which was dissolved in a 1:1 benzene-heptane mixture, the solution was treated with charcoal and cooled. Yield, 25.5 Gm (66%); m. p. 78–79°.

Anal.—Calcd. for $C_{11}H_{11}NO$: N, 8.09. Found: N, 7.83.

7,8 - Benzo - 1,3 - diazaspiro[4.5]decane - 2,4-dithione (Compound S.2-816).—A solution made up of 17.3 Gm. (0.1 mole) of 2-tetralone cyanohydrin, 8 Gm. of carbon disulfide, 10 cc. of ethanol, previously saturated with ammonia, and 20 cc. of benzene and contained in a pressure bottle was allowed to remain at room temperature for six days. The mixture was filtered, the filtrate was concentrated, and the residue was dissolved in ether. The ether was extracted with dilute sodium hydroxide, and the alkaline extract was acidified to precipitate the crude product which was recrystallized from dilute isopropyl alcohol. Yield, 2.4 Gm. (9%); m. p. 208–209°.

Ethyl α - Cyano - α - 3,4 - dihydro - 2(1H) - naphthylidineperen acetate.—A mixture of 24.5 Gm. (0.17 mole) of 2-tetralone, 27.1 Gm. (0.24 mole) of ethyl cyanoacetate, 9.6 Gm. of glacial acetic acid, 3 Gm. of ammonium acetate, and 100 cc. of benzene was refluxed under a Dean-Stark water separator for three and one-half hours. An additional 3 Gm. of ammonium acetate was added, and refluxing was continued for another four hours. The mixture was cooled, water was added, and the layers were separated. The benzene layer was washed with saturated sodium chloride, dried, and concentrated to an oil which was distilled. Yield, 28.5 Gm. (69.5%); b. p. 178–180° (2 mm.).

2 - Carboxy - 1,2,3,4 - tetrahydro - 2 naphthaleneacetic Acid.—To a stirred solution of 28.5 Gm. (0.118 mole) of the naphthylidine acetate in 90 cc. of ethanol at room temperature was added a solution of 15.6 Gm. (0.24 mole) of potassium cyanide in 90 cc. of water. After being refluxed for 15 minutes the mixture was cooled, acidified, and extracted with benzene. The benzene solution was distilled to a viscous amber residue which was dissolved in a mixture of 100 cc. of glacial acetic acid and 200 cc. of 36% hydrochloric acid. The solution was refluxed for 46 hours, distilled to remove most of the acetic acid, and the residue was diluted with water to precipitate an oil. The oil solidified, and the solid was recrystallized from a mixture of chloroform and heptane using charcoal to decolorize. Yield, 20 Gm. (73%); m. p. 139–140°.

Anal.—Calcd. for $C_{13}H_{14}O_4$: Neut. equiv. 117. Found: Neut. equiv. 117.

7,8 - Benzo - 2 - azaspiro[4.5]decane - 1,3 - dione (Compound S.2-823).—A mixture of 8.2 Gm. (0.035 mole) of 2-carboxy-1,2,3,4-tetrahydronaphthaleneacetic acid and 8 cc. of 28% aqueous ammonia was distilled to a solid residue. Heating was continued, and at about 260° the solid melted, and gas was evolved from the melt. The temperature of the

liquid was gradually raised to 300°, after which the cooled viscous material was dissolved in isopropyl alcohol. The solution was treated with charcoal, diluted with water, and the solid which separated was recrystallized from dilute acetone. Yield, 4.6 Gm. (61%); m. p. 233–235°.

Anal.—Calcd. for $C_{12}H_{12}NO_2$: N, 6.51. Found: N, 6.33.

The corresponding 2-methyl derivative (Compound S.2-771) was prepared in a similar fashion using methylamine instead of ammonia and a reaction temperature of 230°; m. p. 72–74° after recrystallization from dilute ethanol.

Anal.—Calcd. for $C_{14}H_{15}NO_2$: N, 6.11. Found: N, 5.98.

7,8 - Benzo - 3 - oxa-1 - azaspiro[4.5]decane - 2,4-dione (Compound S.2-840).—A three-neck flask, fitted with a stirrer and two dropping funnels, was charged with a solution of 19 Gm. (0.09 mole) of 2-amino-1,2,3,4-tetrahydro-2-naphthoic acid monohydrate in 100 cc. of 1 *N* sodium hydroxide. By means of the funnels, 28.3 Gm. (0.26 mole) of ethyl chlorocarbonate and 250 cc. of 1 *N* sodium hydroxide were added simultaneously to the stirred solution at 25°, at such a rate that both funnels emptied at the same time (20 minutes). The mixture was stirred at 25° for three hours, acidified, and extracted with ether. The dried ether solution was distilled to a solid residue to which 30 cc. of thionyl chloride was added, and after the initial reaction had subsided, the mixture was warmed on a steam bath for one-half hour and distilled to remove the excess thionyl chloride. The solid residue was recrystallized twice from benzene-petroleum ether mixtures, using charcoal to decolorize. Yield, 16.7 Gm. (84%); m. p. 150–151°.

Anal.—Calcd. for $C_{12}H_{11}NO_3$: N, 6.45. Found: N, 6.46.

N - Methyl - 1,2,3,4 - tetrahydro - 1,2 - naphthalene dicarboximide (Compound S.2-787).—The temperature of a mixture of 8.9 Gm. (0.04 mole) of 1,2,3,4-tetrahydro-1,2-naphthalene-dicarboxylic acid (25) and 14.9 Gm. (0.12 mole) of 25% aqueous methylamine was gradually raised to 225–230° and maintained for three hours, allowing the water formed to distill from the mixture, and the mixture was then distilled under reduced pressure. Yield, 7.6 Gm. (88%); b. p. 150–153° (0.4 mm.).[5]

Anal.—Calcd. for $C_{13}H_{13}NO_2$: N, 6.51. Found: N, 6.44.

7,8 - Benzo - 1 - oxa - 3 - azaspiro[4.5]decane-2,4-dione (Compound S.2-733).—2-Hydroxy-1,2,3,4-tetrahydro-2-naphthonitrile (24.3 Gm., 0.14 mole) was added to a solution of 36 Gm. of 96% sulfuric acid in 50 Gm. of 95% ethanol, and the mixture was refluxed for 18 hours, after which it was cooled and poured onto ice. The oil was extracted with ether and distilled to obtain 16.7 Gm. (54%) of ethyl 2-hydroxy-1,2,3,4-tetrahydro-2-naphthoate boiling at 118–122° (0.5–1 mm.).

The ester and 4.8 Gm. (0.08 mole) of urea were added to a sodium ethylate solution prepared from 2.0 Gm. (0.086 gram atom) of sodium and 50 cc. of ethanol. The mixture was refluxed for two hours, cooled, diluted with water, and extracted with ether. Acidification of the aqueous solution precipitated the solid oxazolidine-dione which was recrystallized from ethanol using charcoal to decolorize. Yield, 9.6 Gm. (58%); m. p. 192–193°.

Anal.—Calcd. for $C_{12}H_{11}NO_3$: N, 6.45. Found: N, 6.35.

5 - Methyl - 5 - (1,2,3,4 - tetrahydro - 1 - naphthyl)hydantoin (Compounds S.2-790 A & B).—A mixture of 16.8 Gm. (0.096 mole) of methyl 1,2,3,4-tetrahydro-1-naphthyl ketone, 9.8 Gm. (0.15 mole) of potassium cyanide, 28.8 Gm. (0.03 mole) of ammonium carbonate, 90 cc. of ethanol and 30 cc. of water was heated at 55–60° for 10 hours. The mixture was diluted with 500 cc. of water, cooled, and filtered. The solid, weighing 18.8 Gm., melted over a wide range beginning at 175° and was separated into two components as follows: it was digested in warm ethanol and filtered from the insoluble solid. The filtrate was diluted with water to precipitate a crystalline solid which was recrystallized from a mixture of chloroform and heptane. Yield, 8.3 Gm., m. p. 184–186°.

The alcohol-insoluble solid was dissolved in dilute sodium hydroxide, the solution was treated with charcoal and acidified. The precipitated solid was recrystallized from acetic acid to obtain 5.8 Gm., m. p. 259–261°.

7,8 - Benzo - 1,3 - diazaspiro[4.5]decane - 4 - one Hydrochloride (Compound S.2-913).—Raney nickel (75 Gm.) (25) was suspended in a solution of 11.5 Gm. (0.05 mole) of 7,8-benzo-1,3-diazaspiro [4.5] decane-4-one-2-thione in 250 cc. of ethanol, and the mixture was stirred and refluxed for two hours and filtered. The filtrate was concentrated to approximately 25 cc., cooled, and the solid was isolated. Yield, 6.1 Gm. (51%); m. p. 172–173° after recrystallization from water. The hydrochloride salt, prepared with ethanolic hydrogen chloride and recrystallized from ethanol, melted with decomposition at 249–250°.

Anal.—Calcd. for $C_{12}H_{15}ClN_2O$: N, 11.73. Found: N, 11.80.

[5] The 6-methoxy derivative has been reported by Crowley, G. P., and Robinson, R., *J. Chem. Soc.*, **1938**, 2001.

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A Study of Warexin,[1] A New Hypochlorous Acid Germicide*

By VELMA L. CHANDLER, ROLLIN E. PEPPER, and LEE E. GORDON

Bactericidal and sporicidal activities of Warexin, a hypochlorous acid germicide, were studied. Warexin in concentrations as low as 0.1 per cent was found to be bactericidal. The presence of proteinaceous material markedly reduced the germicidal potency and a 1.25 per cent solution was not sporicidal. Warexin was found to be corrosive for the types of stainless steel used for manufacturing fine surgical instruments.

HYPOCHLORITES are effective germicides by virtue of their powerful oxidizing properties. However, their widespread use is restricted because hypochlorites react with organic matter resulting in a reduction of their germicidal effectiveness. The potency is likewise related to *p*H and may be markedly affected by the alkalinity or acidity of the conditions of sterilization.

Hypochlorites have been used in the dairy and food industries as a sanitizing agent for processing equipment. Such solutions have also found their use in medicine and public health. Recently there have appeared several reports on the use of Clorpactin®,[2] the trade name for a group of hypochlorous acid compounds, in the treatment of tuberculous cystitis (1), as a surgical antiseptic (2), as a tuberculocide (3), and as a topical germicide in otolaryngology (4).

The present communication is concerned with the results of tests of Warexin, a Clorpactin-containing compound, with special reference to its applicability to chemical sterilization in hospitals.

MATERIALS AND METHODS

Reagents.—Warexin-powdered material was obtained in 5-oz. quantities in brown bottles with a plastic screw top fitted with a specially designed plastic insert to effect a tight seal. Solutions of varying concentrations were prepared by adding weighed amounts of Warexin to distilled water.

Microorganisms.—*Clostridium tetani, Clostridium sporogenes,* two unidentified aerobic bacilli (Bacillus species E-594 and Bacillus species E-601), *Proteus vulgaris, Pseudomonas aeruginosa, Shigella dysenteriae, Mycobacterium tuberculosis* H37Rv, *Candida albicans,* and *Coccidioides immitis* were used.

Spore suspensions of the two Clostridia and the two aerobic bacilli were made by washing six-day-old Fortified Brain Heart Infusion Agar or Trypticase Soy Agar cultures incubated at 37°C. The suspensions were diluted in saline to a final concentration of 1×10^6 spores (heat resistant) per ml.

Twenty-four-hour cultures of *Pseudomonas aeruginosa, Shigella dysenteriae,* and *Proteus vulgaris* grown in Eugonbroth (BBL) at 37°C. were diluted with sterile distilled water to contain 1×10^6 cells per ml.

Mycobacterium tuberculosis H37Rv was grown in Dubos medium for 10 days and 1 ml. was added to 100 ml. of test solution. A three-day culture of *Candida albicans* grown in liquid Sabouraud medium at 25°C was diluted in sterile water to contain 1×10^6 cells per ml. Arthrospores of *Coccidioides immitis,* suspended in sterile water in the concentration of 1×10^6 spores per ml. were used.

Test Procedures.—Two tests were performed. One was a modification of the procedure reported by Portner, *et al.* (5), and employs a Millipore membrane filter on which the test microorganisms are collected and washed free of bacteriostatic material prior to subculture. The other test method was described by Friedl (6) for evaluating sporicidal activity.

In the first technique a Swinney filter adapter which will accommodate a $^1/_2$-inch Millipore filter was used. The Swinney filter adapter containing the membrane filter was connected to the barrel of a cotton plugged 10-ml. syringe and autoclaved. The sterile assembly was connected to a vacuum

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The authors are grateful for the assistance and advice given by Dr. A. A. Stonehill and Mr. John Neumeyer of the Department of Pharmaceutical Chemistry, Research Division Ethicon, Inc.
[1] Trademark of the Guardian Chemical Corp., New York, for its cold sterilizing agent containing Clorpactin®.
[2] Chlorpactin®, brand of monoxychlorosene of the Guardian Chemical Corp., New York.

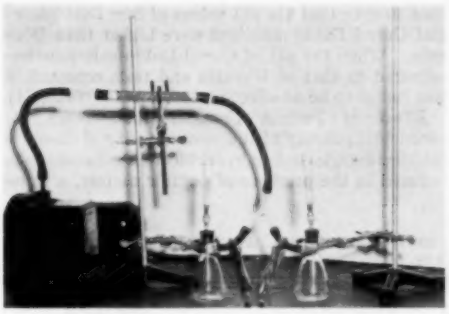

Fig. 1. Apparatus for disinfectant testing.

flask by attaching the end of the Swinney filter adapter to a cannula previously pushed through a rubber stopper in a 250-ml. suction flask. A drying tube inserted into the vacuum line prevented moisture from entering the pump. Figure 1 shows an assembly ready for use.

Briefly, the procedure was as follows: Microorganisms were mixed with the test solution. The final concentration of microorganisms in the mixture was 1,000 cells per ml. After varying exposure times at 22°C., 5 ml. of the mixture was pipetted into the syringe barrel and vacuum applied. When approximately 0.5 ml. of the mixture remained in the syringe, one or two 10-ml. aliquots of water were added to the syringe. When filtration was completed the vacuum was clamped off and the membrane aseptically removed and transferred to subculture media. (Care was exercised not to allow air to pass through the filter membrane during filtration.) Subcultures for anaerobes were made in fluid thioglycollate medium I containing 0.1% soluble starch. Eugonbroth was used for the aerobic gram-positive spore-forming and gram-negative bacilli; Dubos broth for *Mycobacterium tuberculosis;* and fluid Sabouraud medium for Candida and Coccidioides. The tests were examined daily for 30 days with the exception of Mycobacteria which were held for 60 days.

The second test method was that of Friedl (6), and is recommended by the U. S. Department of

TABLE I.—COMPARATIVE GERMICIDAL EFFECT OF WAREXIN, SUN DIAL BLEACH, AND CARREL-DAKIN

			Warexin pH 6.75–7.28 Exp. in Min.					Sun Dial Bleach pH 8.71–9.47 Exp. in Min.				Carrel-Dakin pH 8.3–8.45 Exp. in Min.				
E No.	Organism Name	Concn. of Solution Tested,[c]	<1	5	10	15	30	<1	5	10	30	<1	5	10	15	30
3	*Cl. tetani* spores	1.25	−[a]	−	−	−	−	..	..	..	..	−	−	−	−	−
		0.25	−	−	−	−	−	+[b]	−	−	−	−	−	−	−	−
		0.1	−	−	..	−	−	+	−	−	−	..	..	..	..	..
		0.01	+	−	−	−	−	..	..	..	..	..	..	..	..	..
58	*Cl. sporogenes* spores	1.25	−	−	−	−	−	..	..	..	..	−	−	−	−	−
		0.25	−	−	−	−	−	..	..	..	..	−	−	−	−	−
		0.1	−	−	−	−	−	..	..	..	..	..	..	..	..	..
		0.01	+	−	−	−	−	..	..	..	..	..	..	..	..	..
594	Bacillus culture spores	1.25	−	−	−	−	−	..	..	..	..	+	−	−	−	−
		0.25	−	−	−	−	−	+	+	+	−	+	+	+	−	−
		0.1	+	+	−	−	−	+	+	+	−	..	..	..	..	..
		0.01	+	+	+	+	+	..	..	..	..	..	..	..	..	..
601	Bacillus sp. spores	1.25	−	−	−	−	−	..	..	..	..	+	−	−	−	−
		0.25	+	−	−	−	−	..	..	..	..	+	−	−	−	−
		0.1	+	−	−	−	−	..	..	..	..	..	..	..	..	..
		0.01	+	+	+	+	+	..	..	..	..	..	..	..	..	..
24	*Pr. vulgaris*	1.25	−	−	−	−	−	..	..	..	..	−	−	−	−	−
		0.25	..	..	..	..	..	..	..	..	..	−	−	−	−	−
		0.1	−	−	−	−	−	..	..	..	..	..	..	..	..	..
		0.01	−	−	−	−	−	..	..	..	..	..	..	..	..	..
39	*Ps. aeruginosa*	1.25	−	−	−	−	−	..	..	..	..	−	−	−	−	−
		0.25	−	−	−	−	−	..	..	..	..	−	−	−	−	−
		0.1	−	−	−	−	−	..	..	..	..	..	..	..	..	..
		0.01	−	−	−	−	−	..	..	..	..	..	..	..	..	..
108	*S. dysenteriae*	1.25	..	..	..	..	..	..	..	..	..	−	−	−	−	−
		0.25	−	−	−	..	−	..	..	..	..	−	−	−	−	−
		0.1	−	−	..	−	−	..	..	..	..	..	..	..	..	..
		0.01	−	−	−	−	−	..	..	..	..	..	..	..	..	..
84	*M. tuberculosis*	1.25	..	..	..	..	..	..	..	..	..	..	..	..	..	..
		0.25	−	−	−	−	−	..	..	..	..	..	..	..	..	..
		0.1	−	..	−	−	−	..	..	..	..	..	..	..	..	..
		0.01	+	+	+	+	+	..	..	..	..	..	..	..	..	..
1025	*C. albicans*	1.25	−	−	−	−	−	..	..	..	..	..	..	..	..	..
		0.25	−	−	−	−	−	..	..	..	..	..	..	..	..	..
		0.1	−	−	..	−	−	..	..	..	..	..	..	..	..	..
		0.01	−	−	−	−	−	..	..	..	..	..	..	..	..	..
1055	*C. immitis*	1.25	..	..	..	..	..	..	..	..	..	−	−	−	−	−
		0.25	−	−	−	−	−	..	..	..	..	+	−	−	−	−
		0.1	−	−	−	−	−	..	..	..	..	..	..	..	..	..
		0.01	+	+	+	+	+	..	..	..	..	..	..	..	..	..

[a] Negative.
[b] Positive.
[c] W/V of Warexin or equivalent in HOCl.

Agriculture, Insecticide Division, for testing disinfectants for sporicidal activities (7). The test is performed with surgical silk suture loops impregnated with bacterial spores which resist constant boiling hydrochloric acid for five minutes. Spores of Bacillus species E-594, *Clostridium tetani* (Tulloch Type 2 NIH), and a strain of *Clostridium sporogenes* obtained from the U. S. Department of Agriculture, Insecticide Division, were used.

cant to note that the *p*H values of Sun Dial Bleach and Carrel-Dakin solutions were higher than Warexin. When the *p*H of Carrel-Dakin solutions was adjusted to that of Warexin and tests repeated, it was found to be as effective as Warexin (Table II).

Effect of Protein Contamination.—Since the germicidal potency of the vast majority of chemical disinfectants, including hypochlorites, is known to be reduced in the presence of organic matter, a simu-

TABLE II.—COMPARISON OF GERMICIDAL EFFECT OF WAREXIN AND CARREL-DAKIN SOLUTION WITH LOWERED *p*H

			Warexin *p*H 6.75–7.28 Exp. in Min.					Carrel-Dakin *p*H 6.58–7.3 Exp. in Min.				
E No.	Organism Name	Concn. of Solution Tested,[c] %	<1	5	10	15	30	<1	5	10	15	30
3	*Cl. tetani* spores	1.25	−[a]	−	−	−	−	−	−	−	−	−
		0.25	−	−	−	−	−	−	−	−	−	−
		0.1	−	−	..	−	−	−	−	−	−	−
		0.01	+[b]	−	−	−	−	..	..	..	..	..
58	*Cl. sporogenes* spores	1.25	−	−	−	−	−	−	−	−	−	−
		0.25	−	−	−	−	−	−	−	−	−	−
		0.1	−	−	−	−	−	−	−	−	−	−
		0.01	+	−	−	−	−	..	..	..	..	..
594	Bacillus culture spores	1.25	−	−	−	−	−	−	−	−	−	−
		0.25	−	−	−	−	−	+	−	−	−	−
		0.1	+	+	−	−	−	+	−	−	−	−
		0.01	+	+	+	+	+	+	+	+	+	+
601	Bacillus sp. spores	1.25	−	−	−	−	−	−	−	−	−	−
		0.25	+	−	−	−	−	+	−	−	−	−
		0.1	+	−	−	−	−	+	−	−	−	−
		0.01	+	+	+	+	+	+	+	+	+	+
24	*Pr. vulgaris*	1.25	−	−	−	−	−	−	−	−	−	−
		0.25	..	..	..	..	..	−	−	−	−	−
		0.1	−	−	−	−	−	−	−	−	−	−
		0.01	−	−	−	−	−	..	..	..	..	..
39	*Ps. aeruginosa*	1.25	−	−	−	−	−	−	−	−	−	−
		0.25	−	−	−	−	−	−	−	−	−	−
		0.1	−	−	−	−	−	−	−	−	−	−
		0.01	−	−	−	−	−	..	..	..	..	..
108	*S. dysenteriae*	1.25	..	..	..	..	..	..	..	..	..	..
		0.25	−	−	−	..	−	..	..	..	..	..
		0.1	−	−	..	−	−	..	..	..	..	..
		0.01	−	−	−	−	−	..	..	..	..	..
84	*M. tuberculosis*	1.25	..	..	..	..	..	..	..	..	..	..
		0.25	−	−	−	−	−	..	..	..	..	..
		0.1	−	..	−	−	−	..	..	..	..	..
		0.01	+	+	+	+	+	..	..	..	..	..
1025	*C. albicans*	1.25	−	−	−	−	−	−	−	−	−	−
		0.25	−	−	−	−	−	−	−	−	−	−
		0.1	−	−	..	−	−	−	−	−	−	−
		0.01	−	−	−	−	−	..	..	..	..	..
1055	*C. immitis*	1.25	..	..	..	..	..	..	..	..	..	..
		0.25	−	−	−	−	−	..	..	..	..	..
		0.1	−	−	−	−	−	..	..	..	..	..
		0.01	+	+	+	+	+	..	..	..	..	..

[a] Negative.
[b] Positive.
[c] W/V of Warexin or equivalent in HOCl.

EXPERIMENTAL

Germicidal Effect.—In the first group of experiments the bactericidal and sporicidal activities of varying concentrations of Warexin were compared with two other hypochlorite solutions prepared to contain equivalent quantities of hypochlorous acid. The results as presented in Table I show that Sun Dial Bleach[1] and Carrel-Dakin solutions were slightly less germicidal than Warexin. It is significant in-use test of Warexin was made. To solutions of Warexin, sterile bovine serum was added to give a final concentration of 5% serum (0.3% serum protein). Test microorganisms were added and after intervals up to 30 minutes of exposure aliquots were passed through Millipore filters. Table III contains the observations made. Warexin in concentrations of 0.25% or lower was less active when serum protein was present.

Germicidal Activity of Aged Solutions.—These results prompted a study of the germicidal effectiveness of Warexin contaminated with serum and aged

[1] Distributed by Twin County Grocers, Inc., Linden, N. J.

TABLE III.—GERMICIDAL TESTING OF WAREXIN WITH 5% BOVINE SERUM AND WAREXIN WITHOUT SERUM

Test Organism	Warexin W/V, %	HOCl for Serum Experiment, %	pH	Exposure Time in Minutes: Warexin Plus 5% Bovine Serum <1	5	10	15	30	Warexin Without Serum <1	5	10	15	30
E-3	1.25	0.0529	7.1	−	−	−	−	−	−	−	−	−	−
Cl. tetani spores	0.25	0.0121	7.35	+	+	+	+	+	−	−	−	−	−
	0.1	0.00504	7.25	+	+	+	+	+	−	−	−	−	−
E-594	1.25	0.0529	7.1	−	−	−	−	−	−	−	−	−	−
Bacillus sp. spores	0.25	0.0121	7.35	+	+	+	+	+	+	−	−	−	−
	0.1	0.00504	7.25	+	+	+	+	+	+	−	−	−	−
E-601	1.25	0.0529	7.1	−	+	−	−	−	−	−	−	−	−
Bacillus culture spores	0.25	0.0121	7.35	+	+	+	+	+	+	−	−	−	−
	0.1	0.00504	7.25	+	+	+	+	+	+	−	−	−	−
E-24	1.25	0.0529	7.1	−	−	−	−	−	−	−	−	−	−
Pr. vulgaris	0.25	0.0121	7.35	−	−	−	−	+	−	−	−	−	−
	0.1	0.00504	7.25	+	+	−	−	−	−	−	−	−	−
E-39	1.25	0.0529	7.1	−	−	−	−	−	−	−	−	−	−
Ps. aeruginosa	0.25	0.0121	7.35	−	−	−	−	−	−	−	−	−	−
	0.1	0.00504	7.25	+	−	−	−	−	−	−	−	−	−

prior to inoculation. Solutions containing 1.0% and 1.25% of Warexin and 0.3% serum protein were prepared and allowed to stand at room temperature (22° C.) for 12 hours. Similar concentrations of Warexin without serum were aged under the same conditions. Serum was added to the latter solutions after 12 hours. All solutions were then inoculated with a spore suspension of Bacillus species E-594 and tested by the Millipore technique. It was found that neither concentration of Warexin which had been aged with serum was sporicidal following exposure of 30 minutes. The aged Warexin solutions contaminated with serum just prior to test were sporicidal (Table IV).

TABLE IV.—WAREXIN NO. 2 AGED TWELVE HOURS WITH AND WITHOUT 0.3% SERUM PROTEIN TESTS[a] WITH SPORES OF E-594 BACILLUS CULTURE

Exposure Period, min.	Serum Added at Hour "0", Spores Added at Hour "12": 1.25% Warexin		1.0% Warexin		Serum Added at Hour "12", Spores Added at Hour "12": 1.25% Warexin		1.0% Warexin	
1	+	+	+	+	−	−	−	−
5	+	+	+	+	−	−	−	−
10	+	+	+	+	−	−	−	−
15	+	+	+	+	−	−	−	−
30	+	+	+	+	−	−	−	−

[a] Duplicate tests performed.

Storageability.—To determine the storageability of crystalline Warexin, samples of 60 grams each were packaged in hermetically sealed polyethylene-aluminum-paper-laminated envelopes. The filled envelopes were stored for 30 days at 70° F., 100° F., and 120° F. under normal conditions of humidity as well as under 92% controlled humidity. Solutions prepared from the material stored at temperatures over 70° F. showed evidence of deterioration when tested with spores of Bacillus species E-594 and *Clostridium tetani* (Table V).

Sporicidal Testing by U. S. D. A. Method.—Additional sporicidal testing was made with dried silk suture loops contaminated with spores of Bacillus species E-594, *Clostridium sporogenes* and *Clostridium tetani*. The loops were immersed in a freshly prepared 1.25% solution of Warexin and after varying times of exposure, were transferred to fluid thioglycollate medium I containing 0.1% soluble starch. These results indicated that a 1.25% solution of Warexin was not sporicidal for exposures as long as two hours.

Corrosiveness.—Since hypochlorite solutions are known to cause corrosion of certain types of stainless steel, it appeared pertinent to examine the corrosiveness of Warexin since the formulation contained an unidentified anticorrosive (8). Samples of stainless steel were immersed in a 1.5% solution for three 8-hour periods, and washed in distilled water after each 8-hour immersion. Table VI shows the types of stainless steel examined. Types C-20 and those of the 300 series were unaffected while the 400 series, with the exception of Type 430, showed slight to considerable rusting.

CONCLUSIONS

Warexin solutions in concentrations as low as 0.1 per cent are highly bactericidal for a variety of microorganisms under laboratory conditions of test. However, in concentrations as high as 1.25 per cent, Warexin is not sporicidal by the test recommended by the U. S. Department of Agriculture. Its effectiveness is markedly reduced by contamination with proteinaceous material and at storage temperatures above 70° F.

In spite of the remarkable spectrum of germi-

TABLE V.—SPORICIDAL ACTIVITY OF SOLUTIONS PREPARED FROM PACKAGED WAREXIN STORED FOR 30 DAYS UNDER DIFFERENT TEMPERATURES AND HUMIDITY

Warexin % W/V	Temp. % Humid HO Cl %[a] pH[a] Exposure, min.	Orig. Test — — 0.0828 7.6		Conditions of Aging — 70° F. Normal 0.0803 7.6	70° F. 92% 0.0699 7.55	100° F. Normal 0.073 7.78	100° F. 92% 0.0766 7.7	120° F. Normal 0.0414 7.6	120° F. 92% 0.0083 7.3
Tests With E-594, Bacillus Culture Spores									
1.25	<1	−	−	−	−	−	−	+	+
	5	−	−	−	−	−	−	−	−
	10	−	−	−	−	−	−	−	−
	15	−	−	−	−	−	−	−	−
	30	−	−	−	−	−	−	−	−
0.25	<1	+	+	+	+	+	+	+	+
	5	−	−	+	−	−	−	+	+
	10	−	−	−	−	−	+	+	+
	15	−	−	−	−	−	+	−	−
	30	−	−	−	−	−	−	−	−
0.1	<1	+	+	+	+	+	+	+	+
	5	−	−	+	−	−	+	+	+
	10	−	−	−	−	−	−	+	+
	15	−	−	−	+	−	+	−	+
	30	−	−	−	+	−	−	−	+
0.01	<1	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+
	15	+	+	+	+	+	+	+	+
	30	+	−	+	+	+	+	+	+
Tests With E-3 *Clostridium tetani*									
1.25	<1	−	−	−	−	−	−	−	−
	5	−	−	−	−	−	−	−	−
	10	−	−	−	−	−	−	−	−
	15	−	−	−	−	−	−	−	−
	30	−	−	−	−	−	−	−	−
0.25	<1	−	−	+	−	+	−	−	+
	5	−	−	−	−	−	−	−	−
	10	−	−	−	−	−	−	−	−
	15	−	−	−	−	−	−	−	−
	30	−	−	−	−	−	−	−	−
0.1	<1	−	−	−	−	+	−	−	+
	5	−	−	−	−	−	−	−	+
	10	−	−	−	−	−	−	−	−
	15	−	−	−	−	−	−	−	−
	30	−	−	−	−	−	−	−	−
0.01	<1	+	+	+	+	+	+	+	+
	5	+	+	+	−	+	−	+	+
	10	+	+	−	−	+	−	−	+
	15	+	+	−	−	+	−	−	+
	30	+	+	−	−	+	−	−	+

[a] HOCl and pH values refer to 1.25% preparation only. Organism controls were satisfactory.

TABLE VI.—CORROSIVE EFFECT OF 1.5% WAREXIN ON STAINLESS STEEL

Type of Stainless Steel	Observation
C-20	No effect
302	No effect
303	No effect
304	No effect
305	No effect
316	No effect
400	Rusting appeared after 30 min.
410	Slight rusting
420	Slight rusting
430	No effect
440B	Considerable rusting
440C	Considerable rusting

cidal potency of this compound it seems unlikely that it has broad application as a chemical sterilizer for use in hospitals and more particularly in operating rooms. It would appear unrealistic to imagine such conditions of use to be without risk of contamination by protein matter. Coupled with this is the undesirable corrosion of the types of stainless steel commonly used for fine surgical instruments.

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A Comparison of the Physiological Activity of Thiophene Isomers*

By E. CAMPAIGNE

For comparative purposes, data on the activity of 2- and 3-substituted thiophene derivatives have been recalculated in terms of the activity and toxicity of the phenyl analog as unity.

THIOPHENE ANALOGS of many physiologically active benzene compounds have been prepared, and have usually been found to have similar activity. Erlenmeyer (1) noted that thiophene could replace benzene in serological reactions, and referred to the "isosteric" relationship between thiophene and benzene to account for this ability. Until recently all data on the thiophene analogs were based on compounds containing the 2-thienyl radical. However, a second series of thiophene analogs of physiologically active benzene compounds can exist, which contains the 3-thienyl radical. In 1946, a convenient synthesis of 3-substituted thiophenes was developed in our laboratories (2). At that time, the possibility of differences between the physiological properties of 2- and 3-substituted thiophene isomers led us to undertake, in cooperation with the Sterling-Winthrop Research Institute, a study of a large variety of 3-thienyl analogs of physiologically active compounds.

During the past ten years, a number of papers have been published in this field (see references), including a few from other laboratories. Sufficient data have now been accumulated to confirm the original hypothesis that significant differences in the activity of these isomers may occur. Table I is an effort to present concisely the activities and toxicities of various thiophene isomers, in multiples of the activity and toxicity of the corresponding benzene analogs. It must be emphasized that the values in this table are approximations only. Wherever possible, an effort was made to obtain direct comparison of the phenyl, 2-thienyl, and 3-thienyl compounds in simultaneous parallel experiments. However, in order to complete the table, many relationships were calculated from reports in the literature, in which compounds were compared on the basis of their activities relative to that of some well-known medicinal agent which may be considered a secondary standard. For example, the data for item 4, the β-arylethylamines, were calculated as follows: the vasopressor action of the 3-thienyl analog was compared directly to β-phenylethylamine in anesthetized dogs, and found to have about one-third the potency. Direct comparison to epinephrine in similar experiments gave ratios of 72–200, with an average value of 132 multiples of the assay dose of epinephrine. Tainter (3) reports the 2-thienyl analog to be active at 531 times the assay dose of epinephrine. One can then estimate that the 3-thienyl isomer is approximately 3 to 4 times as active as 2-thienylethylamine. Similarly, the antispasmodic activities in item 3 were calculated with reference to the activities compared to atropine. Item 10 cannot be related to the activity of phenylalanine; therefore the ratio of activities of the two thiophene isomers is presented. In item 12, the figures are multiples of the secondary standard, isonicotinic hydrazide, since data for the phenyl analog were not available (16).

Examination of Table I reveals the following facts. There are six 3-thienyl isomers which are more active than the coresponding 2-thienyl compounds (items 2, 4, 5, 10, 11, 12). For example, in the antihistamine series, the 3-isomer is about 5 times as active, while in the antitubercular pair, the 3-isomer is about 20 times as active as the 2-isomer. There are no cases in which the 2-isomer is significantly more active, but in five examples (items 1, 3, 6, 7, 9) activities are essentially equal. In two cases the toxicities are significantly different: the 3-thienyl antihistamine is about twice as toxic as the 2-isomer, while the 3-thienylhydantoin (item 5) is only about $^1/_9$ as toxic as the 2-isomer. In one case (item 8) there seems to be a qualitative difference between the 3-thienyl and the phenyl and 2-thienyl derivatives. Blicke and Zienty (12) reported 5-(2-thienyl)-5-ethylbarbituric acid to have about $^4/_5$ the hypnotic activity and $^3/_4$ the toxicity of phenobarbital. The 3-thienyl isomer caused excitement followed by sedation, but no hypnosis, at the same dosage. At twice the dosage, the same effects were observed. The absence of hypnotic activity in dosages of the 3-thienyl isomer which cause characteristic sedation is remarkable. The plant hormone series

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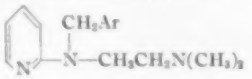
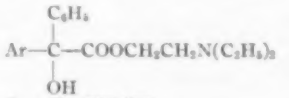
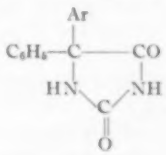
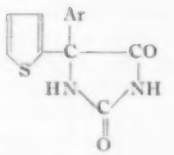
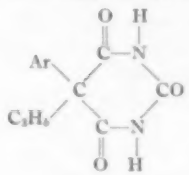
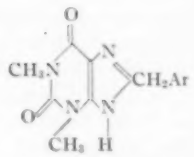
TABLE I.—RATIO OF ACTIVITY AND TOXICITY OF 2- AND 3-THIENYL ISOMERS TO PHENYL ANALOGS OF PHYSIOLOGICALLY ACTIVE COMPOUNDS

Item	Type	Formula	...	Ar = 2-Th.	Ar = 3-Th.	Ref.
1	Local anesthetic	$ArCOO(CH_2)_3N(C_4H_9)_2$	Act.[a]	0.67[b]	0.73[b]	(4)
			Tox.[c]	1.0	0.9	
2	Antihistaminic		Act.[d]	2	5–10	(5, 6)
			Tox.[e]	0.7	1.4	
3	Antispasmodic		Act.[f]	1.3	1.0	(7, 8)
4	Vasoconstrictor	$Ar—CH_2CH_2NH_2$	Act.	0.10	0.3–0.4	(9)
5	Anticonvulsant		Act.[g]	0.30–0.38[h]	0.9	(10)
			Tox.	4.5	0.5	
6	Anticonvulsant		Act.[i]	0.8–2.0[h]	2.0	(10)
			Tox.	0.5	0.75	
7	Anticonvulsant	$Ar—CH_2CONHCONH_2$	Act.	0.10	0.10[j]	(11)
8	Hypnotic		Act.	0.8	(0.8)[k]	(12, 13)
9	Vasomotor (blood press. lowering)		Act.[l]	0.2	0.2	
10	Antiphenyl-alanine	$Ar—CH_2CHCOOH$ (NH$_2$)	Growth Yeast	(1)	(2)[m]	(14, 15)
			Rat	(1)	(1.6)	
11	Plant hormone	$Ar—CH_2COOH$	Act.[n]	0	2.1	
12	Antitubercular	$CH_3—C(=N—NHCSNH_2)—CH=CH—Ar$	Act.[o]	0.008	0.15	(16)

[a] ED_{50} phenyl/ED_{50} thienyl (in this case compared to Butyn,® the *p*-aminophenyl analog.)
[b] Duration of anesthesia, 1% soln. rabbit cornea.
[c] LD_{50} phenyl/LD_{50} thienyl.
[d] Spasmolytic, guinea pig uterine strip.
[e] LD_{50} subcutaneous.
[f] Standard = % atropine activity.
[g] Electroshock, ED_{50} × Dilantin.®
[h] First figure mouse, second rat. Data on 2-thienylhydantoins kindly furnished by Dr. L. M. Long, Parke-Davis and Co.
[i] Compared to Thiantoin.
[j] Anticonvulsant tests by Eli Lilly and Co., courtesy of T. P. Carney.
[k] Sedation only, at same dosage as causes hypnosis in 2-isomer.
[l] Data courtesy G. P. Hager, University of Maryland School of Pharmacy. In dogs, thienyl isomers inhibit respiration, 2-thienyl more toxic than 3-thienyl in this respect, both qualitatively different from the phenyl analog.
[m] Times the activity of the 2-isomer.
[n] Data courtesy R. W. Leeper, Pineapple Research Institute of Hawaii. 2-Thienylacetic acid inactive, phenyl 66% and 3-thienyl 141% of naphthaleneacetic acid in the split pea test.
[o] Compared to the activity of isonicotinic hydrazide against *M. tuberc.* H37RV.

(item 11) represents an example of a qualitative difference between the 2-thienylacetic acid and its 3-thienyl and phenyl analogs.

From the above observations, it can be concluded that it is impossible to predict the activity of a 3-thienyl isomer from a knowledge of the activity of a corresponding 2-thienyl isomer or phenyl analog. Furthermore, in testing thiophene analogs of pharmacologically active benzene derivatives, the 3-isomer should always be included, since there is high probability that it will be active, and a good chance that it will be more active than the 2-isomer. At present, the reasons for significant differences in the activities of the thiophene isomers are obscure. However, these differences may arouse some interesting speculations as to the mode of action of the various compounds.

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Studies on Disintegration of Compressed Tablets I.*

Effect on Disintegration Time of the Procedure used in Incorporating the Disintegrating Agent

By A. DAMODARAN NAIR† and V. N. BHATIA‡

The effects of adding different proportions of the disintegrating agent before and after granulation in a tablet formula have been studied. The sulfathiazole tablets with Veegum as the disintegrator appear to have most rapid disintegrating time when most of the Veegum is added after granulation and only a small proportion before granulation. The U. S. P. tablet disintegration test and a modified U. S. P. tablet disintegration test have been used to measure disintegration time.

DURING the last few years several people have studied the various aspects of tablet disintegration. However, a survey of literature shows that there has been very little done to determine the effect on disintegration time of the procedure used in adding the disintegrating agent to the tablet formula. In the manufacture of tablets, the disintegrating agent may be added to the rest of the ingredients at three different steps: (*a*) before granulation, (*b*) after granulation, (*c*) a portion before granulation and the remainder after granulation.

The work of Higuchi, *et al.* (1), shows that individual granules retain their integrity when they are compressed into tablets. Therefore, it may be assumed that the disintegration of tablets takes place in two steps; first the tablet breaks down to the granules and then the granules break down to smaller particles. (This, of course, would not hold true in those cases where extremely hard tablets are made using very high compression or when very soft granulation is used as this would inevitably lead to excessive crushing of the granules.) Therefore, the amounts of disintegrating agent added before and after granulation may have specific bearing on the rate of disintegration during the respective steps. Gross and Becker (2) in their study of disintegrating agents added all the agent before granulation in one series of tablets, and 95 per

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† Present address: State University of Iowa, College of Pharmacy, Iowa City, Iowa.
‡ Assistant Professor of Pharmacy, State College of Washington, School of Pharmacy, Pullman.

cent before and 5 per cent after granulation in another series. They found the disintegration time was less in tablets made by the second method using starch as the disintegrating agent. Endriss and Melton (3) state that in some tablets it is expedient to add one-fourth of the disintegrating agent (starch) to the original mixture before granulation and the remainder to the granules just before compression. On the other hand, Silver and Clarkson (4) say that very often the starch is divided into two portions, and the majority added to the powdered formula prior to granulation, the remainder being mixed with the lubricant. Incorporated in this manner, the starch serves a double purpose; the portion added to the lubricant rapidly breaks down the tablet into granules, and the starch intimately mixed with the active ingredients disintegrates the granules into smaller particles.

The objective of this investigation was to determine the effect of the three procedures for adding disintegrating agents to a tablet formula. This has been carried out by preparing several batches of sulfathiazole tablets using Veegum[1] as the disintegrating agent and adding it in different proportions before and after granulation. The choice of Veegum was based on preliminary experiments to determine a suitable and convenient formula for the tablets used in this study. The disintegration time for each batch of tablets was then determined.

The size of the granules to be used in tableting usually varies between 12 and 20 mesh (3). In the series of tablets in this investigation, the size was kept at 14 mesh. In the U. S. P. disintegration test (5) the end point is the passage of the particles of the disintegrated tablet through a No. 10-mesh screen. Therefore, it was felt the use of this test alone would not be sufficient in view of the hypothesis regarding the two stages in the breaking of a tablet (tablet to granules and granules to smaller particles). In order to get a better picture from the results, it was decided to use the U. S. P. disintegration test as well as a modified U. S. P. test using a No. 20-mesh screen. It was assumed that the U. S. P. test gave the time taken by the tablets to break up into granules and the modified U. S. P. test the time taken by the tablets to break into granules and then the granules to break up into smaller particles.

EXPERIMENTAL

Sulfathiazole tablets of 0.2 gram were made using acacia gum mucilage (10% w/w) as the binder, Veegum as the disintegrating agent, and stearyl alcohol as the lubricant. The following is the formula for 1000 tablets:

Sulfathiazole	200.0 Gm.
Acacia gum mucilage	58.4 Gm.
Veegum	40.0 Gm.
Stearyl alcohol	A quantity equal to 1% of the dried granules

The proportion of Veegum added before and after granulation is shown in Table I. The total amount of Veegum added in all batches was kept constant and represents 20% of the weight of sulfathiazole. The amount of acacia gum mucilage was enough to keep the granules sufficiently hard so that they would not be crushed into powder when compressed into tablets. Sulfathiazole and Veegum added before granulation were passed through a No. 20-mesh screen and mixed thoroughly. The wet mass obtained after the addition of acacia gum mucilage was forced through a No. 8-mesh screen and dried at 45° for 24 hours. The granules obtained were again forced through a No. 14-mesh screen and collected on a No. 40-mesh screen.

TABLE I.—PROPORTION OF VEEGUM ADDED BEFORE AND AFTER GRANULATION IN THE PREPARATION OF THE TABLETS

Batch Number	Proportion of Disintegrating Agent Added after Granulation, %	Proportion of Disintegrating Agent Added before Granulation, %
1	100	0
2	87.5	12.5
3	75	25
4	62.5	37.5
5	50	50
6	37.5	62.5
7	25	75
8	12.5	87.5
9	0	100
10	0	0

Enough of the particles that passed through the No. 40-mesh screen were returned to the granules in order to keep the amount of "fines" constant at 10%. The remaining portion of Veegum, after passing through a No. 20-mesh screen, was added to the granules and mixed well. The lubricant, stearyl alcohol, previously passed through a No. 100-mesh screen, was also mixed well with the granules. The granules were then compressed in a Stokes "Eureka" single-punch, hand-operated machine using $^{3}/_{8}$ inch standard concave punches. The hardness of the tablets as measured by a Strong-Cobb tablet-hardness tester was maintained between 4 and 5. This degree of hardness was found to be adequate to produce sufficiently hard and firm tablets from the above formula. These tablets were comparable to good commercial tablets in that they did not chip or break in handling. The weight variation of the tablets was ±5 mg. All variables except proportions of Veegum added before and after granulation were kept constant in all batches. The disintegration times of all batches of tablets were determined by the U. S. P. disintegration apparatus and a

[1] Veegum is a complex colloidal magnesium aluminum silicate made by the R. T. Vanderbilt Co., Inc.

modified U. S. P. disintegration apparatus. All stipulations of the U. S. P. test were followed in these determinations with the exception of the use of a No. 20-mesh instead of a No. 10-mesh screen in the modified U. S. P. disintegration apparatus.

RESULTS

The disintegration time of the various batches of tablets as obtained by the U. S. P. tablet disintegration test are shown in Table II and Fig. 1.

TABLE II.—DISINTEGRATION TIME OF TABLETS AS MEASURED BY U. S. P. DISINTEGRATION APPARATUS[a]

Batch Number	Disintegration Time in Seconds		
	Minimum	Maximum	Average
1	26	36	30
2	39	51	48
3	59	98	87
4	90	155	115
5	141	159	151
6	147	220	180
7	196	355	269
8	270	370	313
9	313	422	362
10[b]			

[a] As specified in U. S. P., a minimum of 6 tablets was used in each determination.
[b] Did not disintegrate in one hour.

The disintegration time of the various batches of tablets as obtained by the modified U. S. P. tablet disintegration test is shown in Table III and Fig. 1. Batch 10 which was prepared without any Veegum did not disintegrate in one hour.

TABLE III.—DISINTEGRATION TIME OF TABLETS AS MEASURED BY MODIFIED U. S. P. DISINTEGRATION APPARATUS[a]

Batch Number	Disintegration Time in Seconds		
	Minimum	Maximum	Average
1	1467	2544	2017
2	70	120	98
3	185	224	200
4	225	232	230
5	241	307	272
6	245	354	288
7	375	405	391
8	423	425	424
9	453	570	508
10[b]			

[a] As specified in U. S. P. (5), a minimum of 6 tablets was used in each determination.
[b] Did not disintegrate in one hour.

DISCUSSION AND CONCLUSIONS

If we assume that the U. S. P. tablet disintegration test shows the rate of disintegration of tablets into granules (as hypothesized in the introduction), then an examination of the results will indicate that this first phase of disintegration proceeds more rapidly in those tablets in which a greater proportion of Veegum was added after granulation; the shortest time being taken by the tablets in Batch 1, which had all the Veegum added to them after granulation. On the other hand, the results obtained by using the modified U. S. P. tablet disintegration test show a different picture. Here we assume that the disintegration time noted represents the time taken in both phases of the disintegration of a tablet (tablet to granules and granules to small particles).

An examination of Table III and Fig. 1 will show that the tablets in Batch 1, which showed the shortest disintegration time in the U. S. P. test, take an abnormally long time in the modified test. A comparison of these two results for Batch 1 tablets

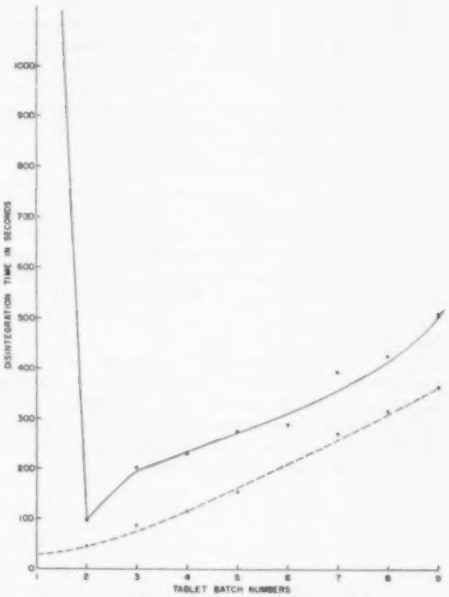

Fig. 1.—Average disintegration time for various batches of tablets. Modified U. S. P. method ——; U. S. P. method - - - -.

would indicate that these tablets disintegrated rapidly into granules, but the granule then took a very long time to break up into smaller particles. This explanation is further supported by the fact that in this batch all the Veegum was added after granulation.

Further examination of the results indicates that the optimum method of adding the disintegrating agent to a tablet formula appears to be the use of a small proportion before granulation and the remainder after granulation. Under the experimental conditions of this investigation and using Veegum as the disintegrating agent, the tablets in Batch 2, in which 12.5% of the total Veegum was added prior to granulation, disintegrated most rapidly.

Another factor which may have some bearing on disintegration time is that an addition of a larger proportion of Veegum or similar disintegrating agents prior to granulation may contribute to an

increased action of the binding agent because of the colloidal nature of these agents and their gel-forming tendencies. This might explain why the increase of disintegration time in batches of tablets in which larger proportions of Veegum have been added before granulation, does not follow a linear pattern (Fig. 1, lower curve), but the curve tends to bend upwards showing that this secondary factor may have further delayed the disintegration time. It may be possible to avoid this by using a nonaqueous, volatile solvent in granulation. This aspect might merit further investigation.

The results also cast some doubt on the usefulness of the U. S. P. disintegration test. It has been pointed out above that the No. 10-mesh screen used in this test has openings larger than the usual size of granules from which tablets are compressed. There seems little, if any, value in measuring the disintegration time if it only refers to the first phase of the breaking up of a tablet. The results of the tests on Batch 1 show that such a measurement can be very misleading. Kelly and Green (6) have pointed out that tablets which behave like those in Batch 1 may be clinically undesirable.

A summary of the literature failed to give any indication as to the rationale for the use of a No. 10-mesh screen in the U. S. P. apparatus. It appears that this screen size was first picked at random, and its use has never been questioned. Many workers have suggested the use of different types of disintegration apparatus (7–13) but they failed to question the validity of the screen size.

In view of the above data, it may be well to reevaluate the U. S. P. tablet disintegration apparatus in reference to the screen size. Since the usual granule size in the tablet manufacture varies between 12–20 mesh, the test would be more valid if a No. 22-mesh or a smaller screen is used.

SUMMARY

1. Sulfathiazole tablets 0.2 Gm. were prepared using Veegum as the disintegrating agent.

2. The Veegum was added in different proportions before and after granulation and the disintegration time noted by two methods.

3. The addition of a small portion of Veegum prior to granulation and the remainder after granulation gave the fastest disintegrating tablets.

4. The data discussed raise a question as to whether a revision of the U. S. P. tablet disintegration test should be considered.

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The Combination of B-Complex Vitamins and Ascorbic Acid in Aqueous Solutions*

By ANA S. GAMBIER and ERWIN P. G. RAHN

A physical and chemical stability study on aqueous solutions of combinations of the B-complex vitamins, with and without vitamin C, has been conducted. The analytical results have been compared after "aging" at room temperature and under accelerated conditions. Satisfactory stability of these vitamins was obtained by observing determined conditions.

A LARGE NUMBER OF PAPERS on the stability of vitamin B-complex and vitamin C aqueous combinations have been published. The stability of vitamins B_2, B_6, niacinamide, and calcium or sodium pantothenate or panthenol, within certain pH limits, has been confirmed in such solutions (1–20). Vitamins B_1, B_{12}, and C are those which have called greater attention to this problem, and, therefore, they constitute the main object of these papers (1–13).

The present investigation, using therapeutic doses, deals with a series of formulations of aqueous solutions of B-complex vitamins with and without vitamin C. Three types of solutions have been studied.

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SOLUTIONS STUDIED

Type A Solutions

B-Complex Solutions Without Vitamin B_{12}.—Few papers report the stability picture of a combination of the most common B-complex vitamins in aqueous solutions (2, 4, 11, 14, 15, 16). Generally one component was studied and only a superficial emphasis was given to the stability of the others (11, 15, 16).

Increasing the concentration of vitamin B_2 results in greater inactivation of vitamin B_1 (Table I, solutions 1 and 5 assayed after 24 m. r. t.[1]). The presence of a greater quantity of air over the solutions also facilitates this oxidative process (19). The reducing action of thiamine and of its decomposition products on riboflavin is denoted by the crystallization of chloroflavin only when high concentrations of vitamin B_1 (15 mg./cc. or more) are used with 0.5 to 1.0 mg./cc. of riboflavin. The precipita-

TABLE I.—TYPE A SOLUTIONS: B-COMPLEX SOLUTIONS WITHOUT VITAMIN B_{12} AT pH 4.5

Soln. No.	Components → Quantities per cc. →	Vit. B_1 mg.	Loss %	Vit. B_2[c] mg.	Loss %	Vit. B_6 mg.	Loss %	Niacinamide mg.	Loss %	Sodium Pantothenate mg.	Physical Changes
1	Theoretical content	6.0	...	0.5	...	1.1	...	26.25	...	3.75	...
	Initial assay	6.04	...	0.52	...	1.05	...	27.7	...	...	...
	After 18 m. r. t.[a]	...	...	...	...	...	...	...	...	...	...[e]
	After 24 m. r. t.	5.37	11.2[b]	0.48	4.0	0.98	6.3[d]	27.6	0	...	...[f]
	Theoretical content	6.0	...	0.5	...	1.1	...	26.25	...	...	...
	Initial assay	6.15	...	0.48	...	1.22	...	28.5	...	...	...
2	After 18 m. r. t.	5.75	4.1[b]	0.47	2.2	1.0	9.0[d]	24.0	8.6[d]	...	...[e]
	After 24 m. r. t.	...	...	...	...	...	...	...	...	...	...[f]
3	Theoretical content	...	...	0.5	...	1.1	...	26.25	...	...	...
	Initial assay	...	...	0.51	...	0.9	...	28.0	...	...	...
	After 18 m. r. t.	...	...	0.5	0	0.85	5.6[d]	25.0	4.8[d]	...	...
	After 24 m. r. t.	...	...	...	...	...	...	...	...	...	...[g]
4	Theoretical content	6.0	...	0.5	...	...	...	26.25	...	...	...
	Initial assay	5.97	...	0.5	...	...	...	26.5	...	...	...
	After 18 m. r. t.	5.63	5.7[b]	0.47	6.0	...	...	26.0	1.0[d]	...	...[e]
	After 24 m. r. t.	...	...	...	...	...	...	...	...	...	...[f]
5	Theoretical content	8.62	...	1.0	...	2.5	...	50.0	...	5.0	...
	Initial assay	8.37	...	1.03	...	2.35	...	52.2	...	...	...
	After 18 m. r. t.	...	...	...	...	...	...	...	...	...	...[h]
	After 24 m. r. t.	6.0	28.4[b]	0.8	20.0	2.45	0	50.0	0	...	...[h]
6	Theoretical content	8.62	...	...	...	2.5	...	50.0	...	5.0	...
	Initial assay	8.25	...	...	...	2.25	...	50.0	...	...	...
	After 18 m. r. t.	...	...	...	...	...	...	...	...	...	...[i]
	After 24 m. r. t.	8.00	3.1[b]	...	...	2.6	0	51.0	0	...	...[j]

Vit. B_1—thiamine hydrochloride.
Vit. B_6—pyridoxine hydrochloride.
[a] m. r. t. = Months at room temperature.
[b] Assayed after separation of the turbidity or precipitate.
[c] Quantities in vitamin B_2, even if different forms of riboflavin were used (18).
[d] Negligible, within the analytical error.
[e] Very slight turbidity after 18 m. r. t.
[f] Slight turbidity after 24 m. r. t.
[g] Without any physical changes over 24 m. r. t.
[h] Heavy precipitation of identified thiochrome, beginning with 6 m. r. t.
[i] Slight turbidity and darkening after 18 m. r. t.
[j] Slight precipitation with darkening at 24 m. r. t.

In this type of solution, vitamin B_1 is rapidly inactivated in the more alkaline range and in the more acidic range riboflavin is precipitated and the calcium or sodium pantothenates show considerable instability. These different characteristics are better harmonized in a pH 4.5 solution. However, the accentuated oxidative action of vitamin B_2 on vitamin B_1 remains, causing the formation of thiochrome, which precipitates quickly (17, 18). The greater the concentration of vitamin B_2 in relation to that of vitamin B_1 in the solution, the quicker and more intensive is the formation of this precipitate (Table I, solutions 1, 2, and 4 compared with 5). tion of thiochrome is observed in every instance before the precipitation of chloroflavin.

These alterations, which very often are within the limits of analytical errors, are more strongly evidenced by physical changes of the solutions. The frequent formation of slight sediments or precipitates, always identified as thiochrome, indicates that such combinations are not stable for long periods of time at room temperature (22 ± 7°). We found that preparations in dosage forms of 2 and 5 cc. containing 6 mg. of vitamin B_1, 0.5 mg. of vita-

[1] m. r. t. = Months at room temperature.

min B_2, 1.1 mg. of vitamin B_6, 26.25 mg. of niacinamide, and 3.75 mg. of sodium pantothenate per cubic centimeter represent the ideal combination of the more common B-complex vitamins in aqueous solutions, as they were free from turbidity up to 18 months at room temperature (Table I, solution 1). This formulation includes a 20% excess of vitamin B_1 and a 25% excess of sodium pantothenate in respect to the amount claimed on the label. The highest purity of the different vitamins, especially of thiamine hydrochloride, must be observed. Assays and stability studies of sodium pantothenate unfortunately could not be made by us.

Type B Solutions

B-Complex Solutions Containing Vitamin B_{12}.—All papers dealing with vitamin B_{12} solutions in the presence of other vitamins of the B group (5–9, 12, 13), refer only to solutions containing low concentrations of vitamin B_{12}, generally 10 to 30 mcg./cc. Current therapeutics employ vitamin B_{12} in parenteral form combined with other components of the B-complex in the treatment of neurological disturbances. Since this is done in massive doses, we studied the behavior of the vitamin B_{12} in concentrations of 500 and 1,000 mcg./cc., combined with vitamin B_1, B_6, and niacinamide in concentrations between 25 and 60 mg./cc.

As Feller and Macek (12) demonstrated unequivocally, the chemical stability of vitamin B_{12} in these combinations depends directly on the presence or absence of decomposition products from the other B group components, especially those containing the thiazolic nucleus originating from the molecule of vitamin B_1. A remarkable parallelism actually exists between the loss of vitamin B_1 and the inactivation of vitamin B_{12} (Table II, solution 8 at pH 3.3 after 3 weeks at 45° and 12 m. r. t.).

An increase of the relative vitamin B_1 concentration promotes correspondent loss of vitamin B_{12} (Table II, solutions 9 and 8 at pH 3.3 or 4.0). The best proportion of concentration between vitamins B_1 and B_{12} in the presence of vitamin B_6 or niacinamide seems to us to be 60 to 1 or less (Table II, solutions 7, 9, and 12).

The pH bearing on the stability of these combinations influences mainly the vitamin B_1, so that the stability of vitamin B_{12} is, within the limits 3.0 to 6.5, indirectly related to it. This explains clearly the behavior of the vitamin B_1, B_6, and B_{12} combination, when submitted to pH values 3.3, 4.0, and 4.5 (Table II, solution 9).

The artificial "aging" test at higher than room temperature levels can also be applied to combinations of vitamin B_{12} with other B-complex components. Its interpretation, however, must be carefully made because the limit of trustworthiness is easily exceeded, causing at times wrong explanations. It is well known that it is impossible to sterilize, without great losses, B-complex parenteral solutions by steam-heating. Depending on the pH, pure vitamin B_1 solutions are equally sensitive to heating. Thus, we prefer not to expose the combination of vitamin B_{12} with other B group components to temperatures of 100° and 120°. The results of such drastic tests, even performed during only 30 to 60 minutes, are no longer an evidence of a "positive compatibility." The formation of decomposition products, especially originating from vitamin B_1, is so increased above room temperature, that concentrations of those decomposition products, which may cause considerable (if not complete) destruction of vitamin B_{12}, are easily reached. Their interference on vitamin B_{12} stability is also directly related to the temperature level to which these solutions are exposed. Thus, the higher the temperature, the greater the inactivation of vitamin B_{12}.

For instance, a vitamin B_1, B_6, and B_{12} combination showed after 12 m. r. t. an 8.3% loss of vitamin B_1 and a 10.6% loss of vitamin B_{12} (Table II, solution 8 at pH 3.3). The same solution heated to 55° during 7 weeks showed a loss of only 5.5% of vitamin B_1 but a 36.1% loss of vitamin B_{12}. Comparatively, the losses after a 45° heating during three weeks in analogous solutions are similar to those obtained after room temperature storage for 12 months (Table II, solutions 7, 8, and 9 at pH 3.3). It seems that the temperature limit for stability studies on aqueous combinations of vitamins B_1, B_6, and B_{12} is 45°.

On the other hand, the artificial "aging" test conditions we used for our vitamin B_1, B_6, and B_{12} combinations were too drastic to prove a possible incompatibility of vitamin B_{12} in a solution containing both vitamin B_1 and niacinamide. Different and irregular results were obtained even at a heating of only 45° after a few weeks. In the case of these solutions we were compelled, therefore, to utilize long tests at room temperature and noticed a loss of only 3.6% of vitamin B_{12} in a solution containing 60 mg. of vitamin B_1, 55 mg. of niacinamide, and 1,000 mcg. of vitamin B_{12} per cubic centimeter at pH 4.5, stored for 12 months (Table II, solution 12).

As this result agrees with those of Macek and Feller (9, 12) and to confirm our "aging" test results, we also performed the other tests of these authors (12) and those of Ponci (13) using a solution which contained 10 mg. of vitamin B_1, 10 mg. of niacinamide, and 25 mcg. of vitamin B_{12} per cubic centimeter. We prepared this solution at pH values 3.3 and 4.5 and submitted it to temperatures of 100° and 120° during 30 and 60 minutes. We actually noticed that vitamin B_{12} losses at 100° were for pH 3.3, 8.5% and for pH 4.5, 10.3% during 30 minutes of exposure and for both pH values 21.1% during 60 minutes of exposure. Nevertheless we found that a 120° heating, even if only for 30 minutes, destroys entirely the vitamin B_{12} in this combination, whatever the pH (Table II, solution 11).

Physical changes in these solutions are also observed. Color changes occur such as darkening and fading. The latter is a neat characteristic of vitamin B_{12} inactivation (Table II, solution 11 after heating up to 100° and 120°). The darkening, as a rule, does not prove the same thing (Table II, solution 12). This we suppose in some cases to be due to color addition between vitamin B_{12} and decomposition products of the other vitamins (20). These changes, which, in spite of the greatest care in handling the solutions and selecting the raw materials, always occur with vitamin B_1, niacinamide and vitamin B_{12} combinations, even at room temperature, are still being studied. To date we have assumed that the origin of these changes is due to the high concentrations of our solutions and to possible influences of the "liquid volume to air volume" ratio in the ampuls or bottles.

TABLE II.—TYPE B SOLUTIONS: B-COMPLEX SOLUTIONS CONTAINING VITAMIN B_{12}

Soln. No.	pH → / Components → / Quantities per cc. →	3.3 ± 0.02 Vit. B_1 mg.	Loss %	Vit. B_6 mg.	Vit. B_{12} mcg.	Loss %	4.0 ± 0.02 Vit. B_1 mg.	Vit. B_6 mg.	Vit. B_{12} mcg.	Loss %	4.5 ± 0.02 Vit. B_1 mg.	Vit. B_6 mg.	Vit. B_{12} mcg.	Loss %
7	Theoretical content	30.0	...	13.75	500	...	30.0	13.75	500	...	...	...	...	...
	Initial assay	...	...	...	485	...	...	...	486	...	...	...	...	...
	After 3 weeks/45°	...	...	...	440	9.3	...	...	451	7.3	...	...	...	...
	After 12 m. r. t.[a]	...	...	...	500	0	...	...	498	0[b]	...	...	...	...
8	Theoretical content	60.0	...	27.5	500	...	60.0	27.5	500	...	...	...	...	...
	Initial assay	59.5	...	28.27	501	...	...	...	485	...	...	...	...	...
	After 3 weeks/45°	58.0	2.5	26.5	478	4.6[b]	...	...	429	11.5[b]	...	...	...	...
	After 7 weeks/55°	56.2	5.5	28.25	320	36.1[c]	...	...	...	...	...	...	...	...
	After 12 m. r. t.	55.0	8.3	26.25	447	10.6[b]	...	...	...	...	...	...	...	...
9	Theoretical content	60.0	...	27.5	1,000	...	60.0	27.5	1,000	...	60.0	27.5	1,000	...
	Initial assay	59.0	...	27.85	1,010	...	...	...	975	...	...	...	960	...
	After 3 weeks/45°	56.5	4.3	27.25	990	1.0	...	...	914	6.2[b]	...	...	881	8.3[b]
	After 12 m. r. t.	57.5	2.6	26.5	1,018	0	...	...	...	...	...	...	...	...

	pH → / Components → / Quantities per cc. →	3.35 ± 0.02 Vit. B_1 mg.	Loss %	Vit. B_6 mg.	Loss %	Vit. B_{12} mcg.	Loss %
10[d]	Theoretical content	2.40	...	1.10	...	22	...
	Initial assay	2.6	...	1.1	...	22	...
	After 3 weeks/45°	2.56	1.6	1.13	0	21.1	4.1

	pH → / Components → / Quantities per cc. →	3.3 ± 0.02 Vit. B_1 mg.	Niacinamide mg.	Vit. B_{12} mcg.	Loss %	4.5 ± 0.02 Vit. B_1 mg.	Niacinamide mg.	Vit. B_{12} mcg.	Loss %
11	Theoretical content	10	10	25	...	10	10	25	...
	Initial assay	..	..	24.5	...	..	..	24.5	...
	After 30 min./100°	..	..	22.4	8.5[e]	..	..	22.0	10.3[e]
	After 60 min./100°	..	..	19.5	21.1[f]	..	..	19.5	21.1[f]
	After 30 min./120°	..	..	<1	>96[g]	..	..	<1	>96[g]
12	Theoretical content	..	..	...	...	60	55	1,000	...
	Initial content	..	..	...	...	..	..	980	...
	After 12 m.r.t.	..	..	...	...	..	..	945	3.6[h]

Vit. B_1—thiamine hydrochloride.
Vit. B_6—pyridoxine hydrochloride.
Vit. B_{12}—crystalline vitamin B_{12} U. S. P.
[a] m. r. t. = Months at room temperature.
[b] Slight darkening.
[c] Accentuated and somewhat irregular darkening.
[d] Formulation for oral product, containing as excipient: glycerin 0.1 cc., soluble saccharin 0.75 mg., methyl *p*-hydroxybenzoate 2 mg., sorbo 0.2 cc., flavors *q. s.*, distilled water *q. s. ad.* 1 cc.
[e] Slight discoloring.
[f] Accentuated uniform discoloring.
[g] Complete discoloring without darkening.
[h] Uniform deep darkening.

Overcoming all these different difficulties, we found that for the vitamin B_1, B_6, and B_{12} aqueous combination the best formulation was the one containing 60 mg. of vitamin B_1, 27.5 mg. of vitamin B_6, and 1,000 mcg. of vitamin B_{12} per cubic centimeter at pH 3.3. In similar concentrations, the vitamin B_1, niacinamide, and vitamin B_{12} combination showed negligible chemical changes, but, the physical changes (darkening) indicate that these solutions are not stable for long periods of time.

Type C Solutions

B-Complex Solutions Free from Vitamin B_{12} and Containing Vitamin C.—The presence of vitamin C in solutions containing vitamins B_1, B_2, B_6, and niacinamide completely displaces the oxidation-reduction equilibrium which exists among the B group vitamins, avoiding thiochrome precipitations and promoting the formation of chloroflavin (18, 19).

Brumfield and Gross (11) conclude that riboflavin suffers a more drastic destruction in this type of solution. Our experience during many years with a product in formulations 13, 14, and 15 (Table III) invariably showed, when maintained at room temperature for longer periods of time, the characteristic crystallization of chloroflavin.

Actually, when these solutions are prepared with riboflavin, the most significant change is the chloroflavin crystallization and the resulting lowering of vitamin B_2 content in the supernatant (Table III, solutions 13, 14, and 15 after 24 m. r. t.). Redissolving the precipitate by heating up to 37° and promoting the reoxidation of the chloroflavin to riboflavin, the total active vitamin B_2 content raised up to 93.4%, as demonstrated by solution 13 (Table III). These findings agree with Siemers' view (4).

The formation of chloroflavin crystals or precipitates is directly influenced by sudden decreases in temperature (from 25° or 37° to +2°) (17, 19). By increasing the proportion of the air in the vials or lowering the riboflavin dosage a delay of these changes is achieved (Table III, solutions 13–15) (17, 19). The use of sodium riboflavin 5′ phosphate permitted the formulation of perfectly stable compositions, both chemically and physically, for long periods of time (Table III, solutions 16 and 17). Only vitamin B_1 gradually loses its activity in these combinations, certainly reinforced under the reducing influence of vitamin C (Table III, solutions 13–16).

Depending on the concentration, pH values lower than 4.6 will provide the gas formation due to vitamin C decomposition. Siemers indicates a minimum pH of 3.8 (4). On the alkaline side the pH again is limited by the greater inactivation of vitamin B_1. We found that a pH between 5.3 and 5.4 was the best for this type of solution.

Temperatures above 37° accelerate excessively vitamins B_1 and C decomposition (Table III, solution 19). The need for a judicious interpretation of the assay results obtained on solutions submitted to more or less intense and prolonged heating is again evidenced in this type of combination. While heating at 45°, during three weeks only, destroys up to 50% of the vitamins B_1 and C, the 37° temperature affords more conclusive results to be related with tests at room temperature. This test performed during 12 months seems to give a picture of the vitamin losses during 36 m. r. t. (Table III, solutions 19 and 18).

There would be a fourth type of solution, i. e., B complex solutions containing vitamin B_{12} and vitamin C. The well known and confirmed incompatibility of vitamin B_{12} with vitamin C (1, 3, 5, 6, 7, 21) leads us not to include this type in our study.

EXPERIMENTAL

All raw materials used showed at least a degree of purity similar to that required by the U. S. P. Special tests "for parenteral use" were performed. The use of buffers or salts for physiologic solutions, as citrates, phosphates, etc., is detrimental to these vitamin combinations. The presence of strange ions must be avoided, especially calcium when vitamin B_1 is present. This is why sodium pantothenate is used instead of the calcium salt. The pH was determined by a pH meter with centesimal readings. The solutions were prepared with fresh double distilled water, at room temperature, in glass containers or all-glass lined apparatus and protected against direct light. For type B solutions, contact with any metal part, even mirror-polished stainless steel of the best quality, must be avoided. The parenteral solutions were sterilized by filtration through Chamberlain L5 microporous porcelain candles and were subdivided under sterile conditions into flame-sealed ampuls of colorless Type I neutral glass.

Assays were made fluorometrically for vitamin B_1 (22) and vitamin B_2 (23), colorimetrically for vitamin B_6 (24), and niacinamide (25), by the spectrophotometric method of Marsh and Kuzel (5), reproduced by Ponci (26), with Beckman DU, for vitamin B_{12} and with Tillmans' reagent for vitamin C (27).

The final sterility of the solutions for parenteral use was bacteriologically confirmed.

Type A Solutions.—Ampuls containing 2 and 5 cc. of solution were prepared. Table I summarizes the results obtained.

The higher the concentration of vitamin B_2, the greater will be the inactivation of vitamin B_1. Vitamin B_2 elimination will make vitamin B_1 completely stable for more than two years. A slight muddling and darkening also in this solution agree with the descriptions in the Pharmacopeia (28). Physical and chemical stability for longer periods than 18 months, can only be obtained in concentrations not exceeding 6 mg. of vitamin B_1 and 0.5 mg. of vitamin B_2 per cc. The use of sodium riboflavin 5′ phosphate or other vitamin B_2 forms does not show any advantage over pure U. S. P. riboflavin (17, 18).

Type B Solutions.—For parenteral use, ampuls containing 1 and 2 cc. were prepared. The oral solution was formulated for daily administration of 10 to 20 cc. Table II summarizes the results obtained.

An increase in vitamin B_1 concentration promotes a proportionate loss of vitamin B_{12}. A pH increase up to 4.0 or 4.5 lowers the stability of vitamin B_{12} as a result, particularly, of decomposed vitamin B_1. If the product-processing rules concerning the preparation of vitamin B_1, B_6, and B_{12} solutions are strictly observed, high dosage combinations, perfectly stable (both chemically and physically) for long pe-

TABLE III.—TYPE C SOLUTIONS: B-COMPLEX SOLUTIONS FREE FROM VITAMIN B_{12} ASSOCIATED TO VITAMIN C AT pH 5.3–5.4

Soln. No.	Components → Quantities per cc. →	Vit. B_1 mg.	Loss %	Vit. B_2 mg.	Loss %	Vit. B_6 mg.	Loss %	Niacinamide mg.	Loss %	Vit. C mg.	Loss %	Liquid to Air Volume Ratio	Physical Changes
13	Theoretical content	5.0	...	0.3[b]	...	0.4	...	10.0	...	55.0	...	1.32:1.00	...
	Initial assay	5.1	...	0.31	...	0.42	...	10.21	...	54.5	...	...	...
	After 24 m. r. t.[a]	3.2	36	0.17	43.3[c]	0.4	0	9.8	2	48.3	11.6	...	...[e]
		...	...	0.28	6.6[d]	...	...	...	...	...	...	...	...
14	Theoretical content	5.5	...	0.24[b]	...	0.4	...	10.0	...	55.0	...	2.82:1.00	...
	Initial assay	4.8	...	0.23	...	0.45	...	9.9	...	53.31	...	...	...
	After 24 m. r. t.	3.0	37.5	0.18	21.8[c]	0.42	0	9.8	1	48.0	10.0	...	...[e]
15	Theoretical content	5.0	...	0.24[b]	...	0.4	...	10.0	...	55.0	...	1.32:1.00	...
	Initial assay	4.9	...	0.25	...	0.44	...	9.53	...	53.25	...	...	...
	After 24 m. r. t.	2.9	42	0.18	28.0[c]	0.4	0	10.0	0	46.2	13.0	...	...[f]
16	Theoretical content	5.0	...	0.47[g]	...	0.4	...	10.0	...	55.0	...	2.12:1.00	...
	Initial assay	5.0	...	0.47	...	0.43	...	9.9	...	53.7	...	...	...
	After 18 m. r. t.	3.9	22	0.44	6.0	0.46	0	10.25	0	49.0	9.0	...	...
17	Theoretical content	5.5	..	0.47[g]	...	0.4	...	10.0	...	55.0	...	2.79:1.00	...
	Initial assay	5.25	...	0.43	...	0.45	...	9.8	...	54.27	...	...	...
	After 12 m. r. t.	4.9	11	0.45	0	0.43	0	9.5	3.1	49.0	10.0	...	...
18	Theoretical content	5.5	...	0.47[g]	...	0.4	...	10.0	...	55.0	...	1.85:1.00	...
	Initial assay	5.23	...	0.44	...	0.5	...	10.25	...	53.78	...	...	...
	After 12 m. r. t.	5.1	2.5	0.44	0	0.42	0	9.6	4	49.0	9.0	...	...
	After 12 m./37°	2.7	49.0	0.43	2.3	0.4	0	9.9	1	47.0	13.0	...	...
19[h]	Theoretical content	10.0	...	1.43[g]	...	2.0	...	20.0	...	120.0	...	...	...
	Initial assay	10.1	...	1.4	...	2.1	...	21.0	...	120.0	...	...	...
	After 1 month/37°	6.6	34	1.4	0	...	...	...	...	111.4	7.2	...	...
	After 3 weeks/45°	5.0	50	1.12	20	...	...	...	...	61.45	48.8	...	...
	After 9 m. r. t.	6.9	31	1.50	0	...	...	...	...	110.2	8.2	...	...

Vit. B_1—thiamine hydrochloride.
Vit. B_6—pyridoxine hydrochloride.
[a] m. r. t. = Months at room temperature.
[b] Riboflavin U. S. P.
[c] Assayed in the supernatant.
[d] Assayed after redissolving the chloroflavin crystals.
[e] Crystallization of identified chloroflavin beginning after 5 to 6 m. r. t.
[f] Crystallization of identified chloroflavin beginning after 10 to 12 m. r. t.
[g] Sodium riboflavin 5′ phosphate corresponding to 0.33, respectively, to 1.00 mg./cc. of riboflavin U. S. P.
[h] Formulation for oral product, containing as excipient: glycerin 0.1 cc., methyl *p*-hydroxybenzoate 2.5 mg., sodium cyclamate 35 mg., soluble saccharin 1.11 mg., flavors *q. s.*, distilled water *q. s. ad.* 1 cc.

riods of time at room temperature, will be obtained. The physical stability of solutions containing vitamin B_1 and niacinamide together with vitamin B_{12} is still being studied.

Type C Solutions.—For parenteral use, ampuls containing 5 cc. were prepared. Daily doses of 3 to 6 cc. were contemplated for the oral solution. Table III summarizes the results obtained.

Solutions prepared with sodium riboflavin 5′ phosphate are physically and chemically stable. Of the amounts claimed on the label an excess of 20–30% of vitamin B_1 and 10% of vitamin C must be used, so that the natural losses on these vitamins during normal storage conditions will be compensated for at least 2 years. Vitamins B_2, B_6, and niacinamide did not show any significant activity loss.

CONCLUSIONS

1. Vitamin C can be used as a stabilizer of B complex solutions.

2. Chemically, vitamin B_{12} is perfectly stable in aqueous combinations with the B complex, even at high concentrations, if a certain pH is maintained and if a certain proportion of concentration and processing rules concerning the preparation is observed.

3. Sodium riboflavin 5′ phosphate is not only a riboflavin but also a chloroflavin solubilizer, without being, however, a stabilizer of the former.

4. With the use of sodium riboflavin 5′ phosphate the content of vitamin B_2 in vitamin B + C combinations can be considerably increased.

5. The artificial "aging" tests, when applied to aqueous solutions of water-soluble vitamins, must be considered critically.

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Antiemetics in the Pigeon*

By A. M. BURKMAN, ARTHUR TYE, and J. W. NELSON

An attempt was made to correlate the known antimotion sickness properties of six drugs in man with their ability to protect against drug-induced emesis in the pigeon. Digitoxin and alkavervir were used as the challenging emetics. Three of the six antiemetics were found to produce an emetic response in the pigeon (cyclizine HCl, dimenhydrinate, and diphenhydramine HCl). The remaining three (chlorcyclizine HCl, chlorpromazine HCl, and meclizine HCl) gave no protection against digitoxin-induced emesis. Chlorcyclizine HCl and meclizine HCl protected the animal against alkavervir-induced emesis. Correlation between man and the pigeon for emetic/antiemetic responses was found to be poor.

THE USE OF laboratory animals for the evaluation of the antiemetic and antimotion sickness drugs has resulted in many interpretive difficulties. It is apparent that the availability of a suitable experimental animal would provide (*a*) a means of screening for antiemetic and antimotion sickness drugs, and (*b*) a possible subject for site and mechanism of action studies. Numerous attempts have been made to relate the protective effect of the antiemetics in man in terms of a similar protective effect in the dog and

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cat (1). The drugs have been tested against emesis produced by "swinging" and by a variety of emetic agents, notably apomorphine. The correlation has proved to be neither clear nor complete. Use of such common laboratory animals as the rat, mouse, guinea pig, and rabbit has been neglected, owing to that characteristic peculiar to all rodents, the inability to vomit. For years the pigeon has been used in several assay procedures which rely specifically on an emetic response, namely, the assay of the cardiac glycosides as originally devised by Hanzlick (2), an assay of the alkaloids of *Veratrum viride* (3), and recently a proposed assay for the alkaloids of *Rauwolfia serpentina* (4). While several notes have been published on the effects of some emetic and protective drugs in the pigeon (5–7), detailed information is lacking. Further, the limited information now available is of such a contradictory nature that few conclusions can be drawn from it.

It is felt that the pigeon as an assay animal for the antiemetic and antimotion sickness drugs has not been investigated to any significant extent. And while it is admitted that there is no *a priori* ground for believing that the pigeon is a "suitable" animal, the frank vomiting characteristics of this bird suggest that a more thorough investigation would indeed be valuable, either in supporting or refuting the contention that here exists a possible assay subject.

EXPERIMENTAL

Experimentation was divided into three areas yielding information regarding: (*a*) the response of the pigeon to intravenously administered known emetics, and the determination of an approximate "ED_{90}" for each; (*b*) the effect of the antiemetics, injected intramuscularly; (*c*) the efficacy of the antiemetics in blocking the pigeons' response to an "ED_{90}" of each of the emetic drugs.

The Animals.—Adult pigeons of both sexes weighing 200 to 550 grams were utilized. In many cases the same bird was used in several experiments. In these instances at least five days were allowed for full recovery from the effects of a previous experiment. Small hematomas which were sometimes formed at the site of i. v. injection were invariably broken down and reabsorbed during the five-day rest period.

The Drugs.—The drugs were each dissolved in appropriate volumes of solvent such that injection volumes were kept at approximately 0.5 cc. for i. v. use, and 0.2 cc. for i. m. use. Solutions older than 24 hours were not used.

Calculation of Dosage.—The percentage of body weight due to feathers was determined. The results agree well with Hanzlick's (2) who maintained that an "error" of 10% existed in the true body weight of the pigeon due to feathers. The gross weight of the bird minus 10% is referred to as the corrected weight. It is upon this corrected weight that all dosage is calculated.

The Experimental Run.—The pigeon was weighed to the nearest gram, injected intramuscularly (breast muscle) with an antiemetic and placed in an individual cage with a floor of clean paper. After one hour the bird was removed from the cage, the axillary region cleansed with tincture benzalkonium chloride 1:1000 and the emetic drug injected via the brachial wing vein. The pigeon was then returned to the cage and observed for one hour. Vomiting within the one-hour period only was considered a positive emetic response. Emesis was judged to have occurred only when crop content was actually expelled.

Response to the Emetic Drugs.—Four series of experiments were performed to determine the effect of varying doses of apomorphine HCl, morphine SO_4, digitoxin, and alkavervir (Table I). Latent Period (L. P.) represents that interval of time (in minutes) from the completed injection to the first ejection of vomitus. "Pecking" is an exaggerated, repetitious, and prolonged back and forth movement of the head and neck.

Response to the Antiemetics.—Six series of experiments were performed to determine the effects, if any, of cyclizine HCl, dimenhydrinate, diphen-

TABLE I.—RESPONSE OF THE PIGEON TO THE EMETICS

Drug	Dose mg./Kg.	Ratio Responses			Average Latent Period for Emesis, Min.
		Retching	Emesis	Pecking	
Apomorphine HCl	0.5	1/6	1/6	3/6	...
	1.0	0/6	0/6	3/6	...
	2.0	1/7	0/7	5/7	...
	Control	0/3	0/3	0/3	...
Morphine SO_4	0.2	0/6	0/6	0/6	...
	1.0	1/6	0/6	0/6	...
	2.0	0/6	0/6	0/6	...
	Control	0/3	0/3	0/3	...
Digitoxin	0.2[a]	12/12	12/12	0/12	8.0
	Control	0/3	0/3	0/3	...
Alkaverir	0.02	3/4	3/4	0/4	...
	0.03[a]	12/12	12/12	0/12	8.0
	0.10	4/4	4/4	0/4	...
	Control	0/3	0/3	0/3	...

[a] Chosen to represent approximate ED_{90}'s.

TABLE II.—RESPONSE OF THE PIGEON TO SEVERAL ANTIEMETICS

Drug	Dose mg./Kg.	Emesis Ratio Response	Other Effects	Average Emetic L.P., Min.
Cyclizine HCl	5.0	2/12	...	...
	7.2	6/12	...	...
	10.5	9/12	...	25.0
	Control	0/3	...	...
Dimenhydrinate	5.0	2/12	...	...
	7.2	8/12	...	...
	10.0	10/12	...	14.3
	15.0	10/12	...	10.5
	Control	0/3	...	...
Diphenhydramine HCl	2.0	3/10	...	...
	5.0	4/10	...	...
	10.0	8/10	...	31.8
	15.0	10/10	...	18.2
	Control	0/4	...	...
Chlorcyclizine HCl	2.0	0/6	...	...
	5.0	0/6	...	...
	10.0	0/6	...	...
	20.0	0/6	...	...
	30.0	1/6	...	...
	Control	0/3	...	...
Chlorpromazine HCl	2.0	0/6	...	...
	5.0	0/6	...	...
	10.0	0/6	...	...
	20.0	0/6	Sedation	...
	30.0	0/5	Sedation	...
	Control	0/3	...	...
Meclizine HCl	2.0	0/6	...	...
	5.0	0/6	...	...
	10.0	0/6	...	...
	20.0	0/6	...	...
	30.0	0/6	...	...
	Control	0/3	...	...

Cyclizine HCl ED_{50}: 7.4 (5.9–9.2) mg./Kg.
Dimenhydrinate ED_{50}: 7.5 (6.0–9.4) mg./Kg.
Diphenhydramine HCl ED_{50}: 4.6 (2.7–7.9) mg./Kg.

hydramine HCl, chlorcylizine HCl, chlorpromazine HCl, and meclizine HCl administered intramuscularly (Table II). Since it was observed that several of the antiemetics demonstrated an emetic effect, the particular experimental series was designed to yield sufficient data for the calculation of a median emetic dose. Emetic responses were quantitated using the Litchfield-Wilcoxon method (8).

The Protective Effect of the Antiemetics Against Digitoxin-induced Emesis.—The protective effect of the three antiemetics producing no untoward reactions (i. e., vomiting) in the pigeon was determined. The challenging emetic was digitoxin, 0.2 mg./Kg. i. v. Three additional experiments were designed to detect a possible protective effect of low doses of the "emetic antiemetics," that is, doses too low in themselves to cause emesis (Table III).

Protective Effect of the Antiemetics Against Alkavervir-induced Emesis.—The challenging emetic was alkalvervir, 0.03 mg./Kg., i. v. (Table IV). Where protection was observed, Median Protective Doses (PD_{50}) were calculated using the method of Litchfield and Wilcoxon (8).

DISCUSSION

Of the four emetics administered to the pigeons, only two were demonstrated to be potent emetics

TABLE III.—PROTECTIVE EFFECT OF ANTIEMETICS AGAINST DIGITOXIN

Drug	Doses mg./Kg.	Protection Ratio Response	Average Emetic L.P., Min.
Chlorcyclizine HCl	2.0	1/6	2.6
	5.0	0/6	6.0
	10.0	0/6	6.0
	20.0	0/6	2.9
	30.0	0/6	6.3
	Control	0/3	..
Chlorpromazine HCl	2.0	0/6	9.0
	5.0	0/6	8.0
	10.0	0/6	4.0
	20.0	0/6	6.6
	30.0	0/5	6.3
	Control	0/3	..
Meclizine HCl	2.0	0/6	5.0
	5.0	0/6	3.0
	10.0	0/6	4.0
	20.0	0/6	8.3
	30.0	0/6	4.0
	Control	0/3	..
Cyclizine HCl	1.0	0/6	5.8
	Control	0/1	..
Dimenhydrinate	1.0	0/6	6.1
	Control	0/1	..
Diphenhydramine HCl	1.0	0/6	6.1
	Control	0/1	..

TABLE IV.—PROTECTIVE EFFECT OF ANTIEMETICS TO ALKAVERVIR

Drug	Dose mg./Kg.	Protection Ratio Response	Average Emetic L. P., Min.
Chlorcyclizine HCl	5.0	2/10	5.2
	7.2	2/10	7.0
	10.0	4/10	11.1
	15.0	5/10	...
	20.0	7/10	...
	Control	0/4	...
Chlorpromazine HCl	2.0	0/6	6.6
	5.0	0/6	4.6
	10.0	0/6	6.3
	15.0	1/6	7.2
	20.0	0/7	4.1
	Control	0/3	...
Meclizine HCl	7.0	5/12	4.6
	10.0	7/12	...
	14.0	9/12	...
	Control	0/3	...
Cyclizine HCl	1.0	0/6	6.0
	Control	0/1	...
Dimenhydrinate	1.0	0/6	5.3
	Control	0/1	...
Diphenhydramine HCl	1.0	0/6	5.3
	Control	0/1	...

Chlorcyclizine HCl PD_{50}: 13.4 (8.9–20.1) mg./Kg.

Meclizine HCl PD_{50}: 8.2 (5.7–11.6) mg./Kg.

(digitoxin and alkavervir). Doses of these emetics were desired that would represent approximate ED_{99}'s. It was intended that this would be the challenging dose of the emetic against which the antiemetics would be tested.

Doses (mg./Kg.) of apomorphine and morphine that invariably produce emesis in man and other animals evoked no such effect in the pigeon. An immediate result of species variation makes itself evident. The peculiar pecking symptom induced by apomorphine is noted, but no attempt at explanation can be made. Of particular interest is the fact that morphine produced no such response. Ordinarily the emetic activity of the two drugs is considered to be qualitatively similar. Apparently pecking activity cannot be correlated with emetic activity, although the factor of dosage may be an important consideration. The observation was made by Koster (6) that apomorphine, 5–10 mg./Kg. i. v. in pigeons elicited no emetic response. He also reported a pecking response. It was felt that perhaps somewhat smaller doses of the drug might stimulate an emetic effect. Results in this instance were negative.

In the pigeon three of the six antiemetics have been shown to be emetics in their own right. These three, cyclizine HCl, dimenhydrinate, and diphenhydramine HCl have been successfully used as antiemetics particularly with regard to motion sickness. Their marked emetic activity in the pigeon was unexpected. It remains true that the emetic response may be in itself one of the symptoms of general toxicity and that vomiting might be observed after administration of toxic doses of many drugs not generally considered to be emetics. Yet further experiments have revealed that the lethal doses of these drugs administered to the pigeon by the i. m. route are 10 to 20 times that dose effectively producing emesis. (Cyclizine LD_{50}: 106 (68.4–164) mg./Kg., dimenhydrinate LD_{50}: >200 mg./Kg., diphenhydramine LD_{50}: 63 (50–80) mg./Kg.)

The possibility that conditioning of the animals was responsible for emesis was discarded. Vomiting was evoked in pigeons which had never been used for experimentation, while no emesis was ever observed in the saline controls.

It is interesting to note that on a mg./Kg. basis the doses observed to produce emesis in the pigeon have produced no such effect in other laboratory animals such as the dog and cat. In doses up to 20 mg./Kg., sedation was reported as the major side effect. Compared to this the ED_{50} of cyclizine HCl and dimenhydrinate in the pigeon were in the neighborhood of 7.5 mg./Kg., of diphenhydramine HCl about 4.5 mg./Kg. Koster (6) originally reported that cyclizine in doses of 10 mg./Kg. i. v. and 75 mg./Kg. *per os* caused vomiting in pigeons. That cyclizine is also a potent emetic by the i. m. route has been substantiated here.

Allusion has already been made to the fact that the protective effect of dimenhydrinate and diphenhydramine in the pigeon has received the attention of two groups of investigators. Of special interest is the fact that the emetic effect of these drugs was not reported. This seems peculiar since the emetic response to these drugs is unmistakable. Moser, *et al.* (7), administered their emetic (digitoxin) 15 minutes after the injection of the antiemetic. Since it has been observed that the vomiting due to dimenhydrinate and diphenhydramine may frequently occur after 15 minutes, it seems likely that any "antiemetic induced" emesis occurring might have unknowingly been considered as the expected response to digitoxin. Similar circumstances may well apply to Camiscasca and Vecchiati's work (5).

Lower doses of these drugs (i. e., subemetic) were administered in an attempt to detect a possible protective effect against digitoxin or alkavervir. None was observed.

The three remaining antiemetics, chlorcyclizine HCl, chlorpromazine HCl, and meclizine HCl produced no marked untoward effect in the pigeon. Moderate sedation was noted with the high doses of chlorpromazine (20–30 mg./Kg.). All three failed consistently to block the emetic response to digitoxin. In fact, there seemed to be a tendency for digitoxin induced vomiting to occur sooner (average latent period was shorter) when one of the antiemetics was administered. The tendency is noted although statistically evaluated information has not been prepared.

Chlorcyclizine HCl and meclizine HCl both exhibited a protective effect against veratrum-induced emesis. Swiss (3) has stated that as yet no drug has been tested which alleviates veratrum-induced emesis. Qualification of this statement now appears in order.

Explanation of mechanisms, even probable mechanisms, of the emetic/antiemetic activity of some of these compounds is a matter for conjecture. It might be tempting to propose subtle differences in the chemoreceptor trigger zone of the pigeon that allows an emetic response to digitoxin but not to morphine or apomorphine. But in truth there is no

particular reason to believe that a CTZ exists (or is active) in the pigeon medulla. Neurological investigation with respect to the pigeon is conspicuously lacking.

While it is held that the site of veratrum emesis is the vagal ganglion nodosum in the cat (9), the assumption that this is also the site of emetic action in the pigeon remains unverified.

An examination of the data presented, *in toto*, reveals that correlative information between the emetic response of the pigeon and the emetic response of man are, for the most part, negative. It cannot be expected then that the application of such information derived from the study of the pigeon, directly to man, can be anything but hazardous.

SUMMARY

1. The suggestion that apomorphine HCl and morphine sulfate, by the parenteral route, evoke no emesis in the pigeon, has been verified.

2. A prolonged pecking response to apomorphine was observed. Morphine elicited no such effect.

3. Three drugs, generally considered to be antiemetics in man have evoked an emetic response in the pigeon. These were:

(*a*) Cyclizine HCl
ED_{50} = 7.4(5.9–9.2) mg./Kg.
(*b*) Dimenhydrinate
ED_{50} = 7.5(6.0–9.4) mg./Kg.
(*c*) Diphenhydramine HCl
ED_{50} = 4.6(2.7–7.9) mg./Kg.

4. LD_{50}'s of these drugs were found to be:
(*a*) Cyclizine HCl
LD_{50} = 106 (68.4–164) mg./Kg.
(*b*) Dimenhydrinate
LD_{50} = >200 mg./Kg.
(*c*) Diphenhydramine HCl
DL_{50} = 63 (50–80) mg./Kg.

5. Nonemetic doses of these three drugs gave no protection against emetic doses of either digitoxin or alkavervir.

6. Chlorcyclizine HCl, chlorpromazine HCl, and meclizine HCl demonstrated no protective effect when challenged by emetic doses of digitoxin. The average latent period for emesis appeared shorter than when digitoxin alone was administered.

7. Protection against alkavervir-induced vomiting was effected by chlorcyclizine HCl and meclizine HCl. Median protective doses were calculated to be 13.4 (8.93–20.1) mg./Kg. and 8.2 (5.7–11.6) mg./Kg., respectively.

8. Chlorpromazine HCl gave no protection against alkavervir-induced vomiting.

9. From the data it is observed that little correlation exists between the effect of emetics and antiemetics in the pigeon and in man.

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Influence of Gum Tragacanth on the Bactericidal Activity of Preservatives*

By PHILIP C. EISMAN, JACK COOPER, and DONALD JACONIA

The presence of gum tragacanth exerts a strong neutralizing effect upon the bactericidal activity of chlorbutanol, the *p*-hydroxybenzoates, and the quaternary ammonium base, benzalkonium chloride. This neutralizing effect has also been observed, although to a much lesser extent, with phenol, phenylmercuric acetate, and merthiolate. These findings have important implications for the pharmaceutical industry in that preserved products containing gum tragacanth or other vegetable gums may become completely devoid of self-sterilizing properties soon after manufacture or upon storage.

DURING AN EXPERIMENT involving the measurement of the self-sterilizing properties of a pharmaceutical jelly preserved with 0.5 per cent chlorbutanol and 1:50,000 phenylmercuric acetate, it was observed that the anticipated bactericidal activity of this product was surprisingly low. This finding suggested an interference of preserv-

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ative action by the gum tragacanth contained in the product, which, if experimentally demonstrated, would have important implications for manufacturers of preserved or self-sterilizing jellies.

EXPERIMENTAL

Several of the more commonly used antibacterial agents were dissolved in 3.0% gum tragacanth, a typical ingredient in pharmaceutical jellies, and tested for their ability to destroy *Staphylococcus aureus*. The preservatives investigated were: chlorbutanol, phenylmercuric acetate, merthiolate, a mixture of methyl- and propyl-*p*-hydroxybenzoates, benzalkonium chloride (Zephiran) and phenol. Essentially, the method involved the contamination of the variously preserved samples of gum tragacanth with a standard number of *Staph. aureus* (approximately one million organisms per ml. of sample) and the determination of survival rates by plating in suitable agar medium at varying intervals of time ranging from 10 minutes to several days. For purposes of comparison, tests were also conducted with similar concentrations of these preservatives dissolved in water instead of 3.0% gum tragacanth solution. All preparations were maintained at approximately *p*H 7.0 with *M*/15 phosphate buffer, which was found by prior tests to exert no growth suppressive effect upon the test organism. Preliminary trials were conducted with the preserved solutions to insure that bacteriostatic concentrations of preservative were not carried over into the plating medium. In the case of the two mercurials tested, 0.03% thioglycollic acid agar was employed; and in the benzalkonium chloride experiment, 50 mg. % Tamol N (1) was incorporated into the nutrient agar.

It is clearly evident from the results shown in Table I that the presence of gum tragacanth markedly reduces the bactericidal activities of chlorbutanol, the *p*-hydroxybenzoates, and benzalkonium chloride, in spite of the presence of presumably effective concentrations of preservative. In these instances, considerable growth occurred following a period of partial reduction of the bacterial population. When evaluated on the basis of 99.9% destruction of the test organisms, it is also apparent that phenol, phenylmercuric acetate, and merthiolate suffer a loss in activity in the presence of the gum; although this effect is considerably less pronounced than in the case of the other preservatives.

In order to determine the extent to which varying concentrations of tragacanth interfere with the bactericidal activity of preservatives, an experiment was performed with 3.0, 1.5, and 0.75% tragacanth solutions containing 0.4% chlorbutanol, and the rate of killing determined by plating contaminated samples at intervals of time. As seen in Table II, there exists a relationship between the bactericidal activity of chlorbutanol and the concentration of the gum contained in the preparation. In the presence of 0.75% tragacanth, the lowest concentration investigated, chlorbutanol, nevertheless, failed to exert adequate preservative action since the growth of the organisms was apparent by the 72nd hour.

The observation that appreciable growth of *Staph. aureus* takes place in samples of "preserved" jellies following a period of partial destruction suggests that the inactivation of preservative by the gum is a gradual process reaching completion, as in the case of 0.4% chlorbutanol, within 48 hours after preparation. The assumption therefore follows that, depending upon the preservative used, stored, uncontaminated samples of preserved jellies may, upon seeding with *Staph. aureus*, possess significantly less bactericidal activity than freshly prepared lots of the same composition. The correctness of this assumption was determined by contaminating a 28-day-old solution of 0.5% chlorbutanol in 3.0% gum tragacanth and plating for survivor counts in the usual manner. Similarly tested were 24-day-old tragacanth preparations containing 1:50,000 and 1:100,000 merthiolate. For reference purposes, freshly prepared jellies of the same compositions were also included in this experiment.

According to the results summarized in Table III, 0.5% chlorbutanol jelly, which in the previous experiment (Table I) was capable of destroying all of the test organisms within three hours, succeeded in killing only 75% of the organisms by the tenth day as a consequence of aging. On the other hand, loss of bactericidal activity of 1:50,000 and 1:100,000 merthiolate dissolved in 3.0% gum tragacanth was not evident after a 24-day ageing period. This result was not entirely surprising in view of the findings of the earlier experiment indicating that freshly prepared solutions of phenylmercuric acetate and merthiolate suffered but little loss of activity when dissolved in gum tragacanth.

DISCUSSION

The data presented in this report clearly show that gum tragacanth, an ingredient commonly employed in the manufacture of pharmaceutical jellies, exerts an appreciable suppressive effect upon the activity of certain preservatives, probably as the result of physical adsorption (2). This effect is undoubtedly of practical importance, particularly with respect to the manufacture of preserved jellies. The preservatives which were only slightly influenced by the presence of the vegatable gum were phenylmercuric acetate, merthiolate, and phenol.

The ability of agar to reduce the activity of quaternary ammonium salts was noted by Sherwood (3) and confirmed by Quisno, Gibby, and Foter (2), who concluded that agar plate tests are inappropriate for evaluating the bactericidal powers of preparations containing this class of compounds. Foter and Nisonger (4) extended these studies by evaluating the germicidal action of semisolid pharmaceuticals containing quaternary ammonium salts and, in view of their findings, stated that no prediction of the clinical effectiveness of such preparations should be made on the basis of agar zone sizes. It is not surprising that the results obtained by us with gum tragacanth are in accord with the observations made with agar; since both substances, being colloidal vegetable gums, possess similar physicochemical properties. As far as we can establish, this is the first recorded instance of the neutralizing effect of a vegetable gum, specifically gum tragacanth, on the antibacterial properties of substances other than the quaternary ammonium bases. It is therefore highly probable that other vegetable gums, such as methocel, carboxymethylcellulose and Irish moss,

TABLE I.—INFLUENCE OF GUM TRAGACANTH UPON THE ACTIVITIES OF VARIOUS PRESERVATIVES

Preservative	Concentration of Gum (%)	Concentration of Preservative	Per Cent Destruction of *Staph. aureus* After 10 Min.	1 Hr.	3 Hr.	4 Hr.	6 Hr.	24 Hr.	2 Days	3 Days	4 Days	7 Days
Chlorbutanol	0	0.5%	55		100	...	100					
		0.4%	28		99.9	...	100	100				
		0.3%	15		78	...	...	100				
		0.1%	48		54	...	...	99.9	100			
	3.0	0.5%	5		100	...	100					
		0.4%	0		64	...	79	89	Gr.[a] (1.1 m)	Gr. (36 m)		
		0.3%	0		0	...	...	Gr. (1.7 m)	Gr. (4.7 m)			
		0.1%	0		Gr. (110 m)	...	Gr. (240 m)	Gr. (206 m)				
Phenylmercuric acetate	0	1:25,000	0	96	...	100	...	100				
		1:50,000	0	0	...	26	...	98.8	100			
	3.0	1:25,000	0	0	...	0	...	98	99.8	...	99.9	100
		1:50,000	0	0	...	0	...	88	99.8	...	99.9	100
Merthiolate	0	1:10,000	0	33	28	...	...	55	...	100		
		1:50,000	7	11	24	...	...	66	...	100		
		1:75,000	...	...	23	...	...	63	...	...	100	
		1:100,000	...	...	27	...	...	72	...	...	100	
	3.0	1:10,000	0	0	0	...	...	0	...	99.3	...	99.9
		1:50,000	0	0	0	...	...	0	...	99.4	...	100
		1:75,000	...	...	0	...	...	0	...	...	99.4	99.9
		1:000,000	...	...	0	...	...	0	...	...	72	99.9
p-Hydroxybenzoates[b]	0	0.2%	100	100	...	100						
		0.1%	5	22	...	99.2	...	100	100			
	3.0	0.2%	94	100	...	100						
		0.1%	0	0	...	92	...	99.8	97.4	...	Gr. (10 m)	
Benzalkonium chloride (Zephiran)	0	1:25,000	100	100	...	100						
		1:50,000	100	100	...	100						
		1:100,000	100	100	...	100						
	3.0	1:25,000	0	84	...	99.9	...	Gr. (1.8 m)	Gr. (19 m)			
		1:50,000	0	0	...	79	...	Gr. (>40 m)				
		1:100,000	0	0	...	Gr. (1.6 m)	...	Gr. (>40 m)				
Phenol	0	0.5%	40		95	100	...	100				
		0.3%	22		32	44	...	100	100			
	3.0	0.5%	21		98	99.9	...	99.9	100			
		0.3%	0		0	0	...	90	98.8			

[a] Gr. denotes growth of the test organism; the accompanying figure, ( ), refers to the number of organisms, in millions per ml. of sample.
[b] Consists of a mixture, by weight, of 66.6% methyl ester and 33.3% propyl ester.

TABLE II.—INFLUENCE OF VARYING CONCENTRATIONS OF GUM TRAGACANTH UPON THE ACTIVITY OF CHLORBUTANOL

Concentration of Gum (%)	Per Cent Chlorbutanol	Per Cent Destruction of *Staph. aureus* After 10 Min.	1 Hr.	4 Hr.	24 Hr.	48 Hr.	72 Hr.	96 Hr.
3.0	0.4	0	0	64	98.8	Gr. (0.6 m)	Gr. (22 m)	Gr. (42 m)
1.5	0.4	9	11	60	97.9	Gr. (0.8 m)	Gr. (4.6 m)	Gr. (21 m)
0.75	0.4	32	9	31	62	66	Gr. (0.8 m)	Gr. (8 m)
0	0.4	32	56	99.6	99.9	100	100	...

TABLE III.—ACTIVITIES OF CHLORBUTANOL AND MERTHIOLATE AFTER "AGEING" IN 3.0 PER CENT GUM TRAGACANTH (ROOM TEMPERATURE)

Preservative (in 3.0% Gum)	Treatment	Per Cent Destruction of *Staph. aureus* After 10 Min.	3 Hr.	24 Hr.	2 Days	3 Days	6 Days	10 Days
Chlorbutanol 0.5%	Stored 28 days before contamination	0	0	0	34	...	39	75
	Contaminated immediately upon preparation	13	100	100				
Merthiolate 1:50,000	Stored 24 days before contamination	0	0	23	99.9	100		
	Contaminated immediately upon preparation	0	0	15	91	99.9	100	
Merthiolate 1:100,000	Stored 24 days before contamination	0	0	11	99.9	100		
	Contaminated immediately upon preparation	0	0	0	93	99.9	100	

also reduce the antibacterial effectiveness of preservatives. Although Foter and his associates have only emphasized the undesirability of using agar media for the testing of antibacterial activity of products containing quaternary ammonium salts, it is apparent from our data that such products as well as those preserved with certain other agents, such as chlorbutanol and the *p*-hydroxybenzoates are *per se* deficient in antibacterial activity if they contain vegetable gums as one of the constituents.

SUMMARY

1. The presence of gum tragacanth, one of the vegetable gums commonly used in the preparation of pharmaceutical jellies, appreciably reduces the antibacterial properties of chlorbutanol, *p*-hydroxybenzoates, benzalkonium chloride, and to a lesser extent that of phenol, merthiolate, and phenylmercuric acetate, in spite of presumably effective concentrations of preservative.

2. There exists a relationship between the bactericidal activity of preservatives and the concentration of gum tragacanth contained in the preparation; the greater the concentration of gum, the less effective is the preservative agent.

3. Evidence is presented which demonstrates that, during storage, preserved jellies undergo a loss of antibacterial activity.

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Notes

A Note on the Antigenic Stability of an Enteric-Coated Oral Respiratory Vaccine*

By F. J. MURRAY, H. W. RITTER, and R. E. BOOKMAN

EXPIRATION DATES for bacterial vaccines are usually designated as eighteen months for liquids and five years if dried so as to contain not more than 1% moisture (1). Working with an enteric-coated oral polyvalent respiratory vaccine,[1] it was found that the moisture could not conveniently and routinely be held to 1%. However, since the product more nearly resembled a dry rather than a liquid vaccine, it seemed desirable to conduct antigenic stability studies to ascertain whether antigenicity is retained after five years, and, if so, to what degree it is retained.

The first experiments were designed merely to give qualitative information, since there was no way to predict whether any antigenicity would be present in batches over five years old.

TABLE I.—ANTIGENICITY OF SIX ORGANISMS IN ONE- AND FIVE-YEAR-OLD VACCINE

Vaccine	Survivors/Totals 1-yr. Tablets	5-yr. Tablets	Culture Controls
D. pneumoniae, type I	20/20	20/20	0/20
D. pneumoniae, type II	19/20	14/20	1/20
D. pneumoniae, type III	17/20	20/20	1/20
D. pneumoniae, type VII	11/20	9/20	0/20
D. pneumoniae, type VIII	15/40	13/40	0/40
K. pneumoniae	20/20	20/20	0/20

White mice weighing 18–20 Gm. were injected by intra-abdominal route with 1.0 ml. of a suspension consisting of a single Oravax® tablet in 5.0 ml. of physiological saline solution. Mice of the same sex were used for a given experiment. Five days later the mice were challenged with 1.0 ml. of the appropriate dilution of an 18-hr. broth culture enriched with blood. Based on culture titration records, each challenge consisted of 10 to 100 times the lethal dose.

Results, shown in Table I, indicate that the six organisms tested were still antigenic after five years. Since the data provide no information on the degree of antigenicity, a second series of experiments was designed to yield more quantitative information. The experimental design was the same as described above, except that in addition to the mice receiving the Oravax suspension, others received tenfold dilutions of this material.

TABLE II.—QUANTITATIVE STUDIES ON ANTIGENICITY OF SIX ORGANISMS IN ONE- AND FIVE-YEAR-OLD VACCINE

	Survivors/Totals 1:10	1:100	1:1000	Culture Controls
D. pneumoniae I				
1-yr. tablets	10/20	13/20	2/20	0/40
5-yr. tablets	16/20	8/20	3/20	..
D. pneumoniae II				
1-yr. tablets	18/20	7/20	2/20	3/40
5-yr. tablets	16/20	11/20	4/20	..
D. pneumoniae III				
1-yr. tablets	18/20	12/20	0/20	1/40
5-yr. tablets	18/20	5/20	0/20	..
D. pneumoniae VII				
1-yr. tablets	4/20	0/20	..	0/40
5-yr. tablets	2/20	1/20	..	0/40
D. pneumoniae VIII				
1-yr. tablets	10/20	8/20	1/20	1/60
5-yr. tablets	9/20	9/20	2/20	..
K. pneumoniae				
1-yr. tablets	11/20	5/20	2/20	0/40
5-yr. tablets	15/20	3/20	3/20	..

Results, Table II, show no significant difference between the one and five-year-old tablets.

Moisture determinations were made on tablets from the batches used in this study. The five-year-old tablets had been stored at refrigerator temperature. Using the method of 24-hr. vacuum, room temperature over P_2O_5, the one-year-old material contained 1.54% moisture as compared to 1.80 and 2.40% for the five-year-old batches. The enteric coating is undoubtedly an important factor in preventing excessive moisture uptake by the tablets.

SUMMARY

1. Enteric-coated tablets containing a polyvalent respiratory vaccine were shown to be as antigenic after five years as one-year-old tablets, using mouse protection tests as an index of antigenicity.

2. The moisture content of tablets stored for five years at refrigerator temperature increased less than 1%.

REFERENCE

(1) Dating Decision Number 10, U. S. Public Health Service, 1947.

* Received July 19, 1956, from the Department of Microbiology, Research Laboratories, The Wm. S. Merrell Company, Cincinnati, Ohio.

Acknowledgment is made to Dr. G. O. Sharp of the Analytical and Control Laboratory for supplying moisture determination figures, and to Miss K. A. Ludwig of the Microbiology Department for assistance with the animal studies.

[1] Oravax, registered trademark of The Wm. S. Merrell Company.

ERRATUM

In the article entitled "A Note on an Improved Iontophoretic Device," by Keith F. Dennison, John W. Schermerhorn, and William H. Bargh, published in the December 1956 issue of This Journal, page 818, the diagram pictured was incorrectly drawn. The circuit should have been described as follows: lead (negative) electrode, milliammeter, 10,000 ohm fixed resistor, 50,000 ohm variable resistor, 45-volt B battery and copper (positive) electrode, all connected in series in the above order.

Book Notices

Die äusseren Heilmittel 1950-1955. By HERMANN v. CZETSCH-LINDENWALD and FREIDRICH SCHMIDT-LABAUME. Springer-Verlag, Berlin, 1956. vii + 149 pp. 15.5 x 23.5 cm. Price DM 19.60.

This text is a supplement to the third edition of "Salben, Puder, Externa" (Ointments, Powders, External Preparations), which was published five years ago. The dual purpose of this supplement is to bring the reader up to date on the more recent developments in external applications, and on the other hand, it is an entity in itself, providing the reader with a review of the field over a period of several years.

The text is subdivided into several chapters starting with ointment therapy. In this division the definition of ointments and their subdivision into the physical characteristics is clearly delineated. A discussion of the effectiveness of the different type ointment bases follows with special emphasis placed on the condition of the skin and the desired purpose of ointment therapy. Consideration is given to such medication as cod liver oil, hormones, and vitamins in ointment form. Practically all medicaments which are used externally are discussed.

The second division deals with powders used in external application. Important factors such as physical constants, sterility, pH values, and incompatibilities are discussed.

The remaining chapters concern other external dosage forms such as: lotions, plasters, cleansing agents (medicated soaps), oils, liniments, syrups, and poultices.

The authors have utilized a great deal of information, giving numerous references from the literature. The index consists of 2 separate divisions, an author's index, and an index based on subjects. The print is clear and easily read. This book is a valuable addition to the library of the pharmacist, pharmacologist, and physician.

Commercial Waxes. 2nd ed. Edited by H. BENNETT. Chemical Publishing Co., Inc. New York, 1956. 688 pp. 14.5 x 22.5 cm. Price $15.

This book is an excellent compilation of technical data and practical applications of waxes. The text contains the following main divisions: natural waxes; manufactured and synthetic waxes; physical properties of waxes and wax compositions; wax technology; waxes in industry; glossary, special tables, and substitutes; and wax formulary. An index is appended. The wax formulary includes 50 pages of cosmetic and medicinal preparations and some dental preparations. The format and type used in the printing of the book are very good, with many tables and figures clearly illustrating the text and making for efficient reference use. This second edition of the book will be welcomed by those familiar with the first edition.

Pharmacology and Therapeutics. 7th ed. By CHARLES SOLOMON and ELIZABETH S. GILL. J. B. Lippincott Company, Philadelphia, 1956. xix + 628 pp. 14 x 21 cm. Price $5.

In the seventh edition of this book, which was formerly titled "Pharmacology, Materia Medica and Therapeutics," Miss Gill has attempted to bring the text material up to date while preserving the format and contributions of the late Dr. Charles Solomon. The book is intended as a textbook, primarily for use in teaching the subject to nurses. A short chapter outlines treatment of emergencies. Appended are: a bibliography of other textbooks, a glossary of important (medical) terms, a table of approximate equivalents, and a good general index. The book is very well suited for its intended purpose. It should be useful also to hospital pharmacists.

Recent Progress in Hormone Research. Vol. 12. Edited by GREGORY PINCUS. Academic Press Inc., New York, 1956. 453 pp. 16 x 23.5 cm. Price $10.

This is the twelfth compilation of the proceedings of the Laurentian Hormone Conference; the eleventh volume was noted in THIS JOURNAL, **45**, 190 (1956). The 14 papers in this volume are grouped under four main headings. Under Hormone Biosynthesis and Metabolism: Nature and metabolism of thyroid hormones; The adrenal medulla and the biosynthesis of pressor amines; Biogenesis of the sterols and steroid hormones; Some aspects of the biogenesis of adrenal steroid hormones; and Enzymatic mechanisms of hormone metabolism. Under Hormones and Metabolism: Influence of steroids on

cerebral metabolism in man; Studies of aldosterone in relation to water and electrolyte balance in man; and Functions of insulin and other regulatory factors in peptide formation by animal cells. Under Pituitary Hormones: Human urinary gonadotropin; Hormonal control of melanin pigmentation; and Pituitary syndromes in man. Under Sex Hormones: Male sex hormone and its role in reproduction: Clinical studies of testicular hormone production; and The mechanism of effect of the ovarian steroids. An author and subject index is appended. This book is important for advanced workers in this field.

The Merck Manual of Diagnosis and Therapy. 9th ed. Merck & Co., Inc., Publications Department, Rahway, New Jersey, 1956. xv + 1870 pp. 11 x 17.5 cm. Price, regular edition, $6.75, deluxe edition $9.

The ninth edition of The Merck Manual contains 378 chapters on the diagnosis and therapy of disease. There are 20 main sections in the text, each thumb-indexed, covering specific fields of practice. A general index also is appended to make this a ready and valuable reference for the physician. Pharmacists will be particularly interested in the more than 1600 prescriptions that are included. These appear in groups pertaining to each of the 20 medical divisions and are given at the end of the section, which makes the thumb-index mark for the next section useful also to find the prescriptions for the preceding section. The latest information on the applications of adrenocortical and related therapy, "tranquilizing" drugs, and antituberculosis compounds are included. Discussions of modern advances in the fields of antibiotics, nutrition, immunization, Rh factor, gamma globulin, and prenatal and postnatal care bring together accepted developments on these subjects. Readily applicable advice is included on medical emergencies, burns, poisoning, and shock.

It is interesting to note that this book which has widespread use among physicians includes the following note at the beginning of the group of eye prescriptions: "In view of the complications which may follow use of contaminated 'eye drops,' all ophthalmic solutions must be buffered and sterilized to conform with U. S. P. XV standards. Solutions in multiple-use vials should contain preservatives and should be discarded 10 days after preparation. The preferred method for sterilization of ophthalmic solutions is by filtration through a Selas 03 filter."

Inclusion of reference values and conversion data round out the valuable compilation of material that make this book a necessary addition to the physician's and pharmacist's library.

Dictionary of Poisons. By IBERT MELLAN and ELEANOR MELLAN. Philosophical Library, New York, 1956. 150 pp. 14.5 x 22 cm. Price $4.75

This book describes a limited number of poisonous substances (arranged alphabetically) and briefly outlines the symptoms of poisoning and gives the antidote and first aid treatment for the patient.

BOOKS RECEIVED

Pharmacognosy. 2nd ed. By ROBERTSON Pratt and HEBER W. YOUNGKEN, JR. J. B. Lippincott Company, Philadelphia, 1956. xii + 694 pp. 15.5 x 23.5 cm. Price $10.

Steric Effects in Organic Chemistry. Edited by MELVIN S. NEWMAN. John Wiley & Sons, Inc., New York, 1956. vii + 710 pp. 15 x 23.5 cm. Price $12.50.

Paper Electrophoresis. By G. E. W. WOLSTENHOLME and ELAINE C. P. MILLER (editors for the Ciba Foundation). Little, Brown and Company, Boston, 1956. xii + 224 pp. 14 x 21 cm. Price $6.75.

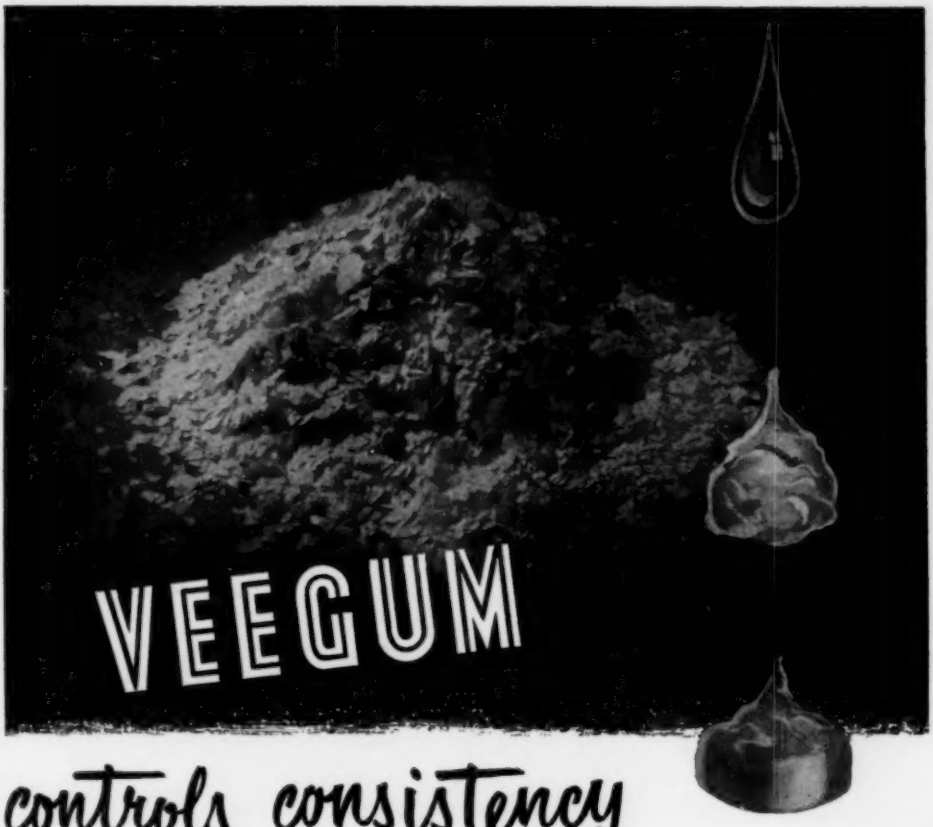
Ageing in Transient Tissues. Vol. II. By G. E. W. WOLSTENHOLME and ELAINE C. P. MILLER (editors for the Ciba Foundation). Little, Brown and Company, Boston, 1956. xi + 263 pp. 14 x 21 cm. Price $6.75.

Lange's Handbook of Chemistry. 9th ed. Edited by NORBERT ADOLPH LANGE and GORDON M. FORKER. Handbook Publishers, Inc., Ohio, 1956. xvi + 1969 pp. 14 x 20.5 cm. Price $8.50.

Histamine. Edited by G. E. W. WOLSTENHOLME and CECILIA M. O'CONNOR (editors for the Ciba Foundation). Little, Brown and Company, Boston, 1956. xvi + 472 pp. 14 x 21 cm. Price $9.

Collectanea Pharmaceutica Suecica. Edited by the Kungl. Pharmaceutiska Institutets Bibliotek, Stockholm. Frenckellin Kirjapaino Osakeyhtiö, Helsinki, Finland, 1956. 56 pp. 15.5 x 23 cm.

Pharmacognosy. 3rd ed. By EDWARD P. CLAUS. Lea and Febiger, Philadelphia Pa., 1956. 731 pp. 18 x 26 cm. Price $12.50



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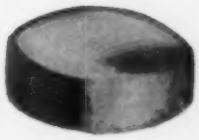

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